Impacts of invasive birds: assessing the incidence and extent of hybridization between invasive Mallard Ducks (*Anas platyrhynchos*) and native Yellow-billed Ducks (*Anas undulata*) in South Africa

by

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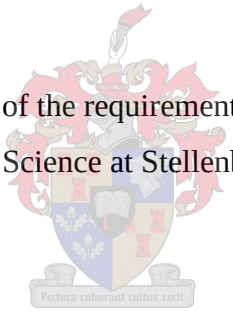

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in the Faculty of Science at Stellenbosch University

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Declaration

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Thesis Abstract

Hybridization with invasive species is a major impact on native congeners, often leading to introgression and the loss of unique genotypes or co-adapted gene complexes. Therefore, hybridization needs to be managed through the removal of invasive species and their hybrids. Such management is often hampered, due to difficulties in identifying hybrids without genetic information, as hybrids are often morphologically indistinguishable from parental species. Consequently, genetic monitoring has become a useful tool in the detection and management of hybridization. Public opposition is often an additional barrier to the control of charismatic invasive species. In the case of the invasive Mallard Duck (*Anas platyrhynchos*) in South Africa, there is strong public opposition to their removal, founded in part, upon a lack of awareness of the potential threat that Mallard Ducks pose to the native Yellow-billed Duck (*A. undulata*) through hybridization and subsequent introgression. Despite this threat, hybridization between the two species is still based on observational evidence with no scientific evidence to support the occurrence and extent of hybridization between these two species.

With this thesis I aim to provide baseline genetic data for the Yellow-billed Duck in South Africa, and use population genetic analyses to determine if there is population genetic structure and differences in genetic diversity measures between widespread native populations. I also aim to determine if hybridization and introgression has occurred and whether sex-biased mating between Mallard and Yellow-billed Ducks occurs in South Africa.

I used nuclear microsatellite DNA markers to determine genetic diversity measures and structure of Yellow-billed Ducks across three populations and historical museum samples from South Africa. The current populations represent a panmictic population with sufficient migration between them to prevent the formation of a strong population genetic structure. This has two implications: firstly, resources do not need to be focused on the conservation of genetically unique populations in South Africa and secondly, that there is the potential for hybridization to spread through long-distance migration between populations. Genetic diversity and structure have not changed significantly over time suggesting that there have been no major changes in the population numbers of Yellow-billed Ducks and their genetic diversity, up until now, is not negatively affected by anthropogenic threats such as urbanisation.

To determine whether hybridization between Mallard and Yellow-billed Ducks, and introgression, have occurred in South Africa I used microsatellite genotype data to identify hybrid individuals and assign them to hybrid genotype classes. There is genetic evidence of

hybridization and introgression between Mallard and Yellow-billed Ducks but most backcrossing and introgression is occurring into the Mallard Duck population. This means that the Yellow-billed Duck population is currently largely unaffected by introgression, but that it may become more extensive in the future. I also used DNA sequencing of a mitochondrial gene region to determine if there was sex-biased mating occurring between these two duck species. This approach revealed that most mating is occurring between Mallard Duck hens and Yellow-billed Duck drakes. These findings indicate that Mallard Duck hens should be prioritised for removal and that it is advisable to remove Mallard Ducks while there is still a chance of protecting the genetic integrity of the Yellow-billed Duck.

These findings have implications for managing hybridization between the two species such as the prioritisation of the removal of Mallard Duck hens and that removal of Mallard Ducks has a good chance of protecting the genetic integrity of the Yellow-billed Duck. Moreover, if these findings are communicated to the public, it may convince them in favour of the removal of invasive Mallard Ducks.

Tesis Opsomming

Hibridisering met indringerspesies is 'n belangrike impak op nabyverwante inheemse spesies, wat dikwels lei tot introgressie en die verlies van unieke genotipes of mede-aangepaste geenkomplekse. Daarom moet hibridisering bestuur word deur die verwydering van indringerspesies en hul basters. Sulke bestuur word dikwels bemoeilik weens probleme met die identifisering van basters sonder genetiese inligting, aangesien basters dikwels nie morfologies van ouerspesies onderskei kan word nie. Gevolglik is genetiese monitering 'n nuttige hulpmiddel in die identifisering en bestuur van hibridisasie. Openbare opposisie is dikwels 'n addisionele versperring vir die beheer van charismatiese indringerspesies. In die geval van indringer Mallardse eende (*Anas platyrhynchos*) in Suid-Afrika, is daar sterk openbare teenkanting teen hul verwydering, wat gedeeltelik gebaseer is op, op grond van 'n gebrek aan bewusmaking van die potensiële bedreiging wat Mallard eende vir die inheemse Geelbekeend (*A . undulata*) inhou deur hibridisasie en daaropvolgende introgressie. Ten spyte van hierdie bedreiging, is hibridisering tussen die twee spesies steeds gebaseer op waarnemingsgetuienis sonder wetenskaplike bewyse om die voorkoms en omvang van hibridisasie tussen hierdie twee spesies te ondersteun.

Met hierdie proefskrif poog ek om basiese genetiese data vir die geelbekeend in Suid-Afrika te verskaf om bevolkingsgenetiese ontledings te gebruik om te bepaal of daar 'n bevolkingsgenetiese struktuur en verskille in genetiese diversiteit tussen wydverspreide inheemse bevolkings is. Ek poog ook om vas te stel of hibridisasie en introgressie plaasgevind het, en of geslags-bevooroordeelde paring tussen Mallardse eende en geelbekeende in Suid-Afrika voorkom.

Ek het kernmikrosatelliet-DNA-merkers gebruik om genetiese diversiteit en struktuur van geelbekeende van drie populasies en historiese museum monsters in Suid-Afrika te bepaal. Die huidige bevolkings verteenwoordig 'n panmiktiese bevolking met voldoende migrasie tussen hulle om die vorming van 'n sterk bevolkingsgenetiese struktuur te voorkom. Dit het twee implikasies: eerstens hoef hulpbronne nie op die bewaring van geneties unieke bevolkings in Suid-Afrika gefokus te word nie. Tweedens, daar is die moontlikheid dat hybridisasie kan versprei deur middel van langafstand-migrasie tussen bevolkings. Genetiese diversiteit en struktuur het nie oor tyd beduidend verander nie, wat daarop dui dat daar geen groot populasiegetal veranderinge in geelbekeende was nie en dat hulle genetiese diversiteit, tot nou toe, nie negatief beïnvloed was deur menslike bedreigings soos verstedeliking nie.

Om vas te stel of hibridisering tussen Mallardse eende en geelbekeende, en introgressie, in Suid-Afrika plaasgevind het, het ek mikrosatelliet genotipe data gebruik om hibried individue te identifiseer en hul aan baster genotipe klasse toe te ken. Daar is genetiese bewyse van hibridisering en introgressie tussen Mallardse eende en geelbekeende, maar die meeste terugkruising en introgressie vind plaas in die Mallardse eend bevolking. Dit beteken dat die geelbekeend bevolking tans grotendeels onaangeraak word deur introgressie, maar dat dit in die toekoms meer prominent kan word. Ek het ook DNA-volgordebepaling van 'n mitochondriale geen-streek gebruik om vas te stel of daar geslags-bevooroordeelde paring tussen die twee eendspesies voorkom. Dié benadering het aan die lig gebring dat die meeste paring tussen Mallardse eend wyfies en geelbekeend mannetjies plaasvind. Hierdie bevindings dui daarop dat wyfie Mallardse eende geprioritiseer moet word vir verwydering en dat dit raadsaam is om Mallardse eende te verwyder terwyl daar nog 'n kans is om die genetiese integriteit van die geelbekeend te beskerm.

Hierdie bevindings het implikasies vir die bestuur van hibridisasie tussen die twee spesies, soos die prioritisering van die verwydering van wyfie Mallardse eende en dat die verwydering van Mallardse eende oor die algemeen 'n goeie kans bied om die genetiese integriteit van die geelbekeend te beskerm. Verder, as hierdie bevindinge aan die publiek oorgedra word, kan dit hulle oortuig ten gunste van die verwydering van indringer Mallardse eende.

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Chapter One

Background

This chapter explores the various mechanisms by which hybridization with invasive species has led to negative impacts on native species across the world. The positive impacts of hybridization are also considered and whether hybridization should in fact be a cause for concern. Management of hybridization is then discussed, both in terms of the various tools and methods available, as well as the barriers that are presented by public opposition. Lastly, the focal issue of this thesis is introduced: the hybridization between Mallard and Yellow-billed Ducks.

Hybridization with non-native species as a threat to native biodiversity

Biological invasions have increased in frequency and scale as a result of accelerated human-mediated movements of species, and are now a major driver of global change (Mack et al., 2000). Invasive species change native communities, resulting in many negative consequences, including: reduced biodiversity, altered ecosystem functioning and even extinction of native species (Mack et al., 2000; Gurevitch & Padilla, 2004; Clavero & Garccıa-Berthou, 2005; Gaertner et al., 2009; McGeoch et al., 2010). Consequently, in order to address biodiversity loss, the identification, control and eradication of invasive species has been made a global target of the Convention on Biological Diversity, an agreement between more than 160 government leaders (CBD; Strategic Goal A, Target 9 of the Aichi Biodiversity Targets: https://www.cbd.int/sp/targets/). Furthermore, invasive species have large economic costs, for example it was estimated that costs associated with invasive species are approximately £1.7 billion/year in the United Kingdom alone (Williams et al., 2010).

Hybridization is ranked as one of the top ten negative impacts of invasive species, ranking fourth in recorded global impacts by invasive birds (Blackburn et al., 2014; Evans, Kumschick, & Blackburn, 2016). Hybridization is the interbreeding of two genetically distinct species and has several mechanisms by which it can threaten native species including: genetic introgression; outbreeding depression; and hybrid vigour (Rhymer & Simberloff, 1996; Echelle & Echelle, 1997; Allendorf et al., 2001; Facon et al., 2005; Hoban et al., 2009; Goodenough, 2010). When hybrid offspring are fertile, with no barrier to gene flow, it can lead to genetic introgression (Rhymer & Simberloff, 1996). Such continuous gene flow between two species through successive backcrossing following hybridization, results in the permanent

incorporation of genes from one species into another (Rhymer & Simberloff, 1996; Wolf, Takebayashi, & Rieseberg, 2001; Schulte, Veith, & Hochkirch, 2012). Populations where introgression has occurred may have various degrees of backcrossing and mating among individuals and can, in some cases lead to complete admixture: the creation of a hybrid swarm where all the individuals in a population contain genetic material from both parental species (Rhymer & Simberloff, 1996; Allendorf et al., 2001; Schulte et al., 2012).

Introgression between non-native species and native congeners results in genetic pollution, and can threaten the native population in several ways, particularly when the native species is already rare or in decline (Ellstrand & Elam, 1993). For instance, the Ethiopian wolf (*Canis simensis*) is outnumbered by domestic dogs (*C. familiaris*) by around 10 to 1, meaning that wolves are more at risk of genetic pollution than dogs (Gottelli et al., 1994). Continued hybridization and introgression can increasingly dilute the genepool of the native species, reducing genetic uniqueness, and in some instances leading to the extinction of native genotypes and co-adapted gene complexes (Ellstrand and Elam 1993; Rhymer and Simberloff 1996; Olden et al. 2004; Schulte et al. 2012). This can result in a loss of the population's locally adapted genotypes, which can reduce the fitness of the population (Ellstrand & Elam, 1993; Johnson, 2000; Allendorf et al., 2001). In the worst case scenario, hybridization and introgression can result in the extinction of the native species (Rhymer & Simberloff, 1996; Mooney & Cleland, 2001).

Introgression is not always extensive; there are pre-zygotic barriers such as mate discrimination and gametic incompatibility, and post-zygotic barriers such as reduced hybrid survival, infertile hybrids, or hybrids that are maladapted to local environmental conditions (Rhymer & Simberloff, 1996; Coyne & Allen Orr, 1998; Storfer, 1999; Furman et al., 2017). These barriers can prevent extensive genetic mixing and introgression. Such lower fitness of hybrids compared to the parental species is known as outbreeding depression and can reduce the fitness of the native population through reduced reproduction success and wasted resources on reproductive effort (Ellstrand & Elam, 1993; Rhymer & Simberloff, 1996; Muhlfeld et al., 2009; Goto, et al., 2011). For example, females of the European Mink (*Mustela lutreola*) hybridize with the non-native American Mink (*M. vison*) but their embryos are not viable (Maran & Henttonen, 1995). Consequently, this reproductive failure can prevent successful reproduction in the European Mink, and contribute to the decline of the European Mink (Maran & Henttonen, 1995), but without the added complications of introgression.

Conversely, in some cases hybrid offspring have a higher fitness than their parental species, a phenomenon known as hybrid vigour (Facon et al., 2005). If hybrids are fertile, interbreeding between hybrids and backcrossing can lead to a positive feedback, increasing the number of hybrids (Allendorf et al., 2001; Fitzpatrick & Shaffer, 2007). Consequently, hybrid vigour can increase introgression and its negative consequences, such as the loss of locally-adapted native genotypes and reduced fitness, and ultimately extinction, of the native population. An example of hybrid vigour is that of hybrids produced between native California cordgrass (*Spartina foliosa*) and invasive smooth cordgrass (*S. alterniflora*) in the saltmarshes of San Francisco Bay (Daehler & Strong, 1997). Here hybrid offspring exceed parental species in many performance traits such as germination, seedling survival and pollen production (Ayres, Strong, & Baye, 2017). Additionally, these cordgrass hybrids are produced primarily through backcrossing and interbreeding between F1 hybrids, consequently, hybrids are increasingly outcompeting the natives and expanding their range (Ayres et al., 2017).

Although there are many negative impacts of hybridization, particularly in the case of hybridization between native and non-native species, hybridization is also an important evolutionary process (Grant & Grant, 1996; Barton, 2001). Hybridization can produce novel genotypes that can facilitate adaption to new ecological niches and facilitate range expansion (Lewontin & Birch, 1966; Grant & Grant, 1996). For example, Lewontin and Birch (1966) investigated whether the Australian fruit fly (*Dacus tryoni*) was able to expand its range to warmer areas due to introgression with *D. neohumeralis*. They found that hybrid individuals were more abundant at higher temperatures, indicating that hybrids were more adapted to elevated temperatures (Lewontin & Birch, 1966). This supported the hypothesis that the adaption to higher temperatures was produced by introgression and allowed the flies to expand their range to new environments (Lewontin & Birch, 1966). Hybridization and introgression can also transfer genes between populations, increasing the rate of evolution by allowing selection to occur on multiple genes instead of one or two alleles produced by mutation (Anderson & Stebbins Jr., 1954; Dowling & Secor, 1997). For instance, Whitney et al. (2006) found evidence that herbivore resistance traits have introgressed from *Helianthus debilis* to *H. annuus annuus*, producing hybrids (*H. annuus texanus*) that have gained adaptive herbivore resistance. Hybridization and introgression can also increase genetic diversity when it occurs between genetically diverse and depauperate populations, which may lead to lower inbreeding depression and increase fitness (Reed & Frankham, 2003; Erickson & Fenster, 2006; Whiteley et al., 2015).

The apparent negative and positive effects of hybridization have led to disagreement about whether, when occurring between native and invasive species, it poses a threat that needs to be prioritised in conservation, mainly because of the grey area surrounding the definition of what a "good" species is (Rhymer & Simberloff, 1996). If a species is defined based on reproductive isolation, i.e. the biological species concept, then hybridization (admixture) could only occur within species. Additionally it is argued that hybridization can have positive effects such as introducing new genotypes and increasing genetic variation; consequently, hybridization should not therefore be a cause for concern (Lewontin & Birch, 1966; Grant & Grant, 1996; Erickson & Fenster, 2006). Conversely, as there is complex genetic variation between populations, genetically distinct populations need to be conserved in order to conserve locally adapted genotypes and the species' ability to adapt to change (Rhymer & Simberloff, 1996; Hobbs & Mooney, 1998; Petit, Mousadik, & Pons, 1998; Reed & Frankham, 2003). Furthermore, even intraspecific hybridization (i.e. admixture) can lead to the loss of local adaptions in populations (Rhymer & Simberloff, 1996; Allendorf et al., 2001). Moreover, when hybridization is the result of human mediated introductions, the protection of anthropogenic hybrids could lead to the extinction of native species and also waste resources on conserving populations of no evolutionary significance (Allendorf et al., 2001). Thus, particularly in the case of hybridization between native and non-native species, hybridization is mostly considered as detrimental and a conservation concern.

Hybridization is often a greater threat when the introduced species outnumbers the native species. Species that are introduced as game species are often characterised by high propagule pressure, and may thus drastically affect the genetic composition of closely related native species through the increased likelihood of hybridization (Bennett et al., 2010; ČíŽková et al., 2012). For example, fish have been introduced across the world as game species and this has resulted in hybridization with native fish species (Rhymer & Simberloff, 1996; Rubidge & Taylor, 2005). The rainbow trout (*Oncorhynchus mykiss*) is one of the most widely introduced fish species, having been introduced to every continent (excluding Antarctica) (Crawford & Muir, 2008; Stanković et al., 2015) and, in many instances, this has resulted in hybridization with narrowly distributed endemics, e.g. the gila trout (*O. gilae gilae*), the apache trout (*O. gilae apache*) and many subspecies of cutthroat trout (*O. clarki*) (Stanković et al., 2015). The high propagule pressure of rainbow trout introductions globally are directly linked to high levels of introgression with native trout species (Bennett et al., 2010). For instance, 65% of all apache trout populations contained individuals harbouring rainbow trout alleles (Dowling &

Childs, 1992). Rainbow trout not only affect the native fish by hybridization, they also compete for space and food (Scott & Irvine, 2007; Seiler & Keeley, 2009). As a result of its various environmental and socio-economic impacts, rainbow trout is listed as one of the worst 100 invasive species in Europe (Nentwig et al., 2018).

Domestic animals and animals introduced as pets also threaten native species through hybridization. For example, wildcats are threatened by hybridization with domestic cats (*Felis catus*) (Hubbard et al., 1992; Oliveira et al., 2008). Habitat fragmentation has led to more contact between domestic and European wildcats (*F. silvestris silvestris*), leading to high levels of admixture in Scotland and Hungary (Beaumont et al., 2001; Pierpaoli et al., 2003; Oliveira et al., 2008). Additionally, cryptic hybrids were identified in Bulgaria, Italy and Portugal (Pierpaoli et al., 2003); Oliveira et al. (2008) thus investigated the ability to detect backcrossing and were only able to detect 85% of backcrossing. This is concerning for the European wildcat species as there is likely to be more admixture than is currently estimated considering the ability to detect backcrossing and evidence of cryptic hybrids (Oliveira et al., 2008).

Introduced amphibians can also have a negative effect on native amphibians through hybridization. The Californian tiger salamander (*Ambystoma californiense*) is able to hybridize with an introduced salamander (*A. tigrinum*). Riley et al. (2003) found significant hybridization amongst their six sample sites, with only three sites containing pure native salamanders and only one site containing more than 8% pure native salamanders. Furthermore, hybrids were found to be viable and fertile, meaning that backcrossing and introgression is possible within these populations (Riley et al., 2003). Leading to the impact of invasive *A. tigrinum* to be listed as massive (Kumschick et al., 2017).

Hybridization can also lead to the evolution of invasiveness; hybrid zones have high levels of genetic variation which can allow for rapid evolution and the creation of novel genotypes (Keim et al., 1989; Ellstrand & Schierenbeck, 2000; Jiggins & Mallet, 2000). These novel genotypes may allow the introduced species to better adapt to their new environment and consequently aid their spread in the introduced range (Ellstrand & Schierenbeck, 2000). For example, the Brazilian pepper tree (*Schinus terebinthifolius*) is an invasive species in Florida that was found to have resulted from at least two separate introductions, each with their own distinct chloroplast haplotype (Williams et al. 2005b). Since their introduction there has been extensive admixture between the two genetic provenances (Williams et al. 2005b) and hybrids have been shown to have greater germination rates, seedling biomass and survival when

compared to the parental individuals (Geiger et al., 2011). Therefore, it is probable that multiple introductions and subsequent admixture facilitated the invasion success of the Brazilian Pepper tree (Mukherjee et al., 2012).

Conservation management of hybridization

There are various methods that conservation managers employ to manage hybridization as a threat to native species including: 1. Release of genetically "pure" native individuals; 2. Public education; 3. Removal of non-native individuals and hybrids; and 4. Genetic monitoring (Hughes et al. 2006; Gese et al. 2015; van de Crommenacker et al. 2015), or 5. A combination of these. For example, for hybridization between the red wolf (*Canis rufus*) and coyotes (*C. latrans*) in the USA, an adaptive management effort was employed that covered several of these methods (Gese et al., 2015). Firstly, red wolves were released from captive breeding programs (Gese et al., 2015). Secondly, a public education program was completed to try to stop members of the public killing the wolves because when a breeding pair was disrupted by mortality, it often led to hybridization (Bohling & Waits, 2015; Gese et al., 2015). Education programs are often needed when there is strong opposition from the public to the control of hybrids and introduced species, as it can help with gaining public support (Bremner & Park, 2007). Lastly, coyotes and hybrids were removed, or sterilised and released and genetic testing was conducted (Gese et al., 2015). The removal of putative hybrids and non-native individuals is the most common method used by conservation managers to manage hybridization (Rieseberg, 1991; Hughes et al., 2006; Robertson et al., 2015). However, hybrid individuals are not always easily identifiable without genetic analysis and thus genetic monitoring is now being used as a tool for early detection and removal of hybrids (Schwartz, Luikart, & Waples, 2006; Devillard et al., 2014; van de Crommenacker et al., 2015).

Conservation genetics as a tool to manage hybridization

Genetic monitoring is a useful conservation tool for managing threatened species particularly as their populations are usually small and fragmented and consequently suffer from lower genetic diversity, reduced fitness and lower adaptive potential (Shaffer, 1981; Lande, 1988; Ellstrand & Elam, 1993). By monitoring population genetic metrics such as effective population size, genetic variation and population structure and migration, conservationists can identify potential threats to a population such as reductions in genetic diversity and gene flow (Allendorf et al. 2001; Schwartz et al. 2006). Additionally, genetic monitoring can be used to

detect hybridization, identify hybrids and determine if introgression is occurring (Schwartz et al., 2006; Vähä & Primmer, 2006).

Molecular genetic analyses are particularly useful for detecting hybridization as it is often difficult to identify F1 and backcrossed hybrids on morphological differences alone, as they can be highly variable (Allendorf et al., 2001; Devillard et al., 2014). Hybrids are generally assumed to be a mix of parental phenotypes but this is not always the case; also, not all morphological variation has a genetic basis meaning that hybrids are often misidentified (Allendorf et al., 2001; Renaud, Alibert, & Auffray, 2012; Devillard et al., 2014). Additionally, if backcrossing occurs, hybrids can have most of their genetic material contributed from one species and can be morphologically indistinguishable from that species (Rhymer & Simberloff, 1996; Devillard et al., 2014). Furthermore, with only observational records, the extent of hybridization and introgression is likely to be underestimated as it is very difficult to determine if a hybrid is a first generation hybrid (F1), a second generation hybrid (F2) or a later generation backcross (Allendorf et al., 2001; Vähä & Primmer, 2006). These distinctions are important in conservation management to make an informed decision about the eradication of hybrids. For instance, to make efficient use of resources it needs to be determined if removing hybrids could allow for population recovery and this is only possible if there are enough pure native individuals, and when significant backcrossing is not occurring (Allendorf et al., 2001). Additionally, if hybrids are not correctly identified the removal of hybrids can potentially result in the loss of unique genotypes and genetic variation (Rhymer & Simberloff, 1996). For example, pallid sturgeon (*Scaphirhynchus albus*) and shovelnose sturgeon (*S. platorynchus*) were recognised as individual species in 1954 (Bailey & Cross, 1954). However, molecular evidence found that pallid and shovelnose sturgeons are not genetically isolated and that interbreeding is likely to be natural (Campton et al., 2000; Tranah et al., 2001). Thus, molecular techniques were able to inform conservation and instead of focusing resources on eradicating hybrids, in this instance, hybrids could be seen as an important evolutionary component of the species (Allendorf et al., 2001).

Public opposition as a barrier to the management of invasive species

Even when using the most effective methods for invasive species management, the success of control efforts is often dependent on public support; public opposition has delayed many invasive species removal programs and has even caused control efforts to stop completely (Bertolino & Genovesi, 2003; van Wilgen & Richardson, 2012; Caetano, 2016). For example, the removal of non-native pine trees (genus *Pinus*) in South Africa received much opposition

from various sectors of public (van Wilgen, 2012; van Wilgen & Richardson, 2012). Despite their known negative impacts on surface run off water and biodiversity in the region (Allan et al., 1997; Le Maitre et al., 2002), the public perceived pines as providing aesthetic, recreational and economic value and considered pines more desirable than the natural and generally treeless habitats of fynbos (van Wilgen, 2012). Another example from South Africa is the ongoing conflict surrounding the issue of trout as invasive species. Brown trout (*Salmo trutta*) and rainbow trout were introduced in South Africa for recreational angling and rainbow trout is now also used for aquaculture (Ellender et al., 2014; Ellender & Weyl, 2014). There is evidence that rainbow trout predate on endemic fish in the Cape Floristic Region; Shelton et al. (2015) found that the mean densities of several native fish were 89-97% lower in invaded streams and trout were found to consume small native Breede redfin (*Pseudobarbus burchelli*). Accordingly, in 2013, rainbow and brown trout were listed as Category 1b invasive species, which indicates that they require control through an invasive species management programme (Government of the Republic of South Africa, 2013). Many stakeholders of the trout industry were strongly opposed to this decision because of concern for the future of the trout industry (Woodford et al., 2017). Additionally, a concern was raised that there was a lack of evidence that trout threaten native biodiversity (Zengeya et al., 2017). Consequently, rainbow and brown trout were removed from the list of alien and invasive species until an agreement could be reached (Woodford et al., 2017).

Such conflicts may be exacerbated for invasive mammals as the "furry animals with big eyes" have charismatic appeal to the general public (Gobster, 2011). For instance, in New Zealand the planned removal of feral horses (*Equus caballus*) in the Kaimanawa area, prompted strong reactions from animal rights activists who opposed the killing of the horses (New Zealand Department of Conservation, 1995; Mack et al., 2000). This resulted in the selling of horses to the public instead of implementing the scientifically based management plan (Mack et al., 2000; Nimmo & Miller, 2007). Only several hundred of the estimated 1500 horses were sold and the management plan was delayed by several years; additionally, damage and threats to native plants by horses still remains an issue (Mack et al., 2000; Linklater et al, 2001; New Zealand Department of Conservation, 2012). This example shows how public opposition can be influential enough to change management decisions when political decisions are prioritised over scientifically based environmental management.

Public opposition can even be the leading cause of failure to eradicate a species; for instance, a management plan in Italy aimed at eradicating the grey squirrel (*Sciurus carolinensis*) was

rendered unsuccessful by public opposition (Bertolino & Genovesi, 2003). The grey squirrel replaces the red squirrel (*S. vulgaris*) in areas where it is introduced and has a negative effect on red squirrel fitness through lower summer breeding and lower recruitment (Gurnell et al., 2004). Consequently, the National Wildlife Institute (NWI) in 1997 proposed an eradication program using live trapping and euthanasia (Bertolino & Genovesi, 2003). Despite the relatively humane methods, there was strong opposition from animal rights activists (McNeely, 2001). The issue was widely publicised, and although public opposition was only from a small group, they utilised the media effectively and their arguments were successful due to the lack of awareness of the public to the issue of invasive animals (McNeely, 2001). The matter ended up in court eventually, delaying the eradication effort by three years, allowing the grey squirrel to expand its range and population to a point where eradication was no longer a feasible management option (Bertolino & Genovesi, 2003).

Hybridization between Mallard and Yellow-billed Ducks

An illustrative example of a non-native species that hybridizes extensively with several closely related native species, mainly within its genus, *Anas*, is the Mallard Duck, *Anas platyrhynchos* (Rhymer, Williams, & Braun, 1994; Fowler, Eadie, & Engilis, 2009; Kulikova, Zhuravlev, & McCracken, 2004; Mank, Carlson, & Brittingham, 2004). Hybridization between Mallard Ducks and various *Anas* species has been assisted by the introduction of Mallards across the world as popular game or ornamental species (Long, 1981; Brooke, 1988; Rhymer, 2006; Champagnon et al., 2009). Mallard Ducks are known to hybridize with: the Florida Mottled Duck (*A. fulvigula*); the American Black Duck (*A. rubripes*); the New Zealand Grey Duck (*A. superciliosa superciliosa*) and the Hawaiian Duck (*A. wyvilliana*) (Rhymer et al. 1994; Mank et al. 2004; Williams et al. 2005a; Fowler et al. 2009). Many of these hybrids are fertile, threatening the genetic integrity of the native ducks through subsequent backcrossing, i.e. genetic introgression (Johnsgard, 1960; Rhymer & Simberloff, 1996). Of particular concern in South Africa is the hybridization between the Mallard Duck and the Yellow-billed Duck, *A. undulata* (Dean, 2000; Owen, Callaghan, & Kirby, 2006; Stafford, 2010). The Mallard Duck was introduced into South Africa in the 1940s and today naturalized populations are widespread in South Africa (Liversidge, 1985; Stafford, 2010). Hybrids between Mallards and Yellow-billed Ducks have been observed in the Eastern Cape, Western Cape, Free State, KwaZulu-Natal and Gauteng Provinces (Brooke, 1988; Dean, 2000; Roberts, 2003; Joubert, 2009). Hybridization between these two species is likely due to the similar calls and resemblance of female Yellow-billed Ducks to female Mallards, making them a likely

alternative mate choice (Skead, 1980; Joubert, 2009). The ability of the two species to produce fertile offspring threatens the genetic integrity/uniqueness of this native duck species (Johnsgard, 1960; Rhymer & Simberloff, 1996; Stafford, 2010). Additionally, Mallards compete with Yellow-billed Ducks for habitats and food, further threatening this native species (Banks et al., 2008). To reduce the potential threat of hybridization, an eradication programme has been introduced; however, there has been a lot of public opposition to their control (Banks et al., 2008; Stafford, 2010). Many residents enjoy feeding Mallards and consider them as pets, and hence the negative reaction to their removal (Stafford, 2010). Additionally, it is believed that one of the main reasons that the public is against Mallard control is because of their lack of awareness of the potential threat that Mallard Ducks pose to the native Yellow-billed Duck (Stafford, 2010).

Aims and Objectives of Study

Examining historical data can be useful for understanding current patterns of genetic diversity (Nielsen, Hansen, & Loeschcke, 1999; Cozzolino et al., 2007; Diedericks et al., 2018). A valuable source of historical genetic data are museum specimens, which can be used to determine historical population structure and genetic diversity (Suarez & Tsutsui, 2004). Additionally, it is important to determine baseline data for the Yellow-billed Duck population in South Africa. By determining baseline genetic metrics, we can establish if the population is vulnerable to hybridization. For instance, a low genetic diversity could indicate lower fitness and competitiveness and therefore a greater risk of Mallards outcompeting Yellow-billed Ducks through hybridization (Fischer, van Kleunen, & Schmid, 2000; Reed & Frankham, 2003). Furthermore, monitoring at all levels of biodiversity (e.g. genes, populations, habitats, etc.) is a vital component to conservation and can identify potential threats to a population such as reductions in genetic diversity and gene flow (Noss, 1990; Hauser et al., 2002; Fruet et al., 2014). Therefore, genetic data could be used to inform the conservation management of the Yellow-billed Duck. Consequently, in Chapter two this research thesis aims: 1) to determine the genetic diversity and structure of a population of Yellow-billed Ducks not occurring in sympatry with Mallard Ducks; and 2) to compare this data to the genetic diversity and population structure of historical Yellow-billed Duck populations.

In South Africa, the perceived hybridization between invasive Mallard and native Yellow-billed Ducks remains based on observation and anecdotal evidence. However, both morphology and molecular analysis are needed to identify hybrids and determine the extent of hybridization and introgression (Rhymer & Simberloff, 1996; Allendorf et al., 2001; Vähä &

Primmer, 2006; Devillard et al., 2014). Furthermore, the lack of a scientific quantification of the extent of hybridization could be contributing to public opposition, because there is a lack of evidence of the threat that Mallard's pose to Yellow-billed Ducks. Therefore, there is an urgent need for a clear scientific quantification of the extent of hybridization using genetic approaches. This information could be used to determine what conservation measures are necessary and how serious the problem of hybridization is between the two duck species, i.e. whether introgression is occurring. Against this background, the aims of Chapter three of this thesis are: 1) to confirm the incidence of hybridization and determine if introgression is occurring and 2) to determine if sex-biased mating is occurring.

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Chapter Two Abstract

Genetic monitoring is a crucial tool to identify potential indicators of threats to a species such as a reduction in genetic diversity. Reduced genetic diversity often translates into reduced fitness and evolvability. Also, archived genetic data such as natural history specimens, are a valuable alternative to long term data sets and can be valuable in improving our understanding of current patterns of genetic diversity.

Potential threats to the Yellow-billed Duck (*Anas undulata*) population in South Africa include hunting, expanding agriculture, and the threat of hybridization with the invasive Mallard Duck (*Anas platyrhynchos*). Hybridization between Mallard and Yellow-billed Ducks has been reported, based on observations, across the majority of the provinces of South Africa, which makes them a significant threat to Yellow-billed Ducks. The threat to the genetic integrity of Yellow-billed Duck, calls for a critical need to obtain baseline genetic data for Yellow-billed Ducks in South Africa.

This study investigates the genetic diversity and structure of three contemporary populations and historical samples of Yellow-billed Ducks. The results show that is a lack of genetic structure and differences in genetic diversity metrics across contemporary populations. There is also evidence of sufficient gene flow between populations to prevent the formation of a strong genetic structure. These populations represent a panmictic population. These finding have two implications. Firstly, the lack of genetic structure across South Africa indicates that current populations can be considered as one conservation unit and so there are no genetically distinct Yellow-billed Duck populations that need to be prioritised for conservation. Secondly, long distance migration between populations indicates that hybrid genes could spread between populations. Additionally, the historical data indicates that there has been no major genetic changes over the last 60 years, suggesting that the Yellow-billed Ducks have not had any major changes in population numbers. This means that Yellow-billed Ducks are not, at least up until now, negatively affected by recent threats such as urbanisation.

Chapter Two

Population genetics reveal panmixia across the South African range of Yellow-billed Ducks (*Anas undulata*)

Introduction

Standing genetic diversity provides the raw material for evolution (Lande & Shannon, 1996). Threatened species often experience low genetic diversity due to threats such as habitat loss, overexploitation and population fragmentation (Frankham, 1996; Spielman, Brook, & Frankham, 2004; Allendorf et al., 2008; Dixo, et al., 2009). Reduced genetic diversity often translates into reduced fitness and evolvability (Lande, 1988; Fischer, van Kleunen, & Schmid, 2000; Reed & Frankham, 2003). Genetic monitoring is thus an essential tool to identify potential indicators of threats to a population such as sudden reductions in genetic diversity or changes in gene flow patterns (Allendorf et al., 2001; Schwartz, Luikart, & Waples, 2006).

There are many examples of ways in which genetic monitoring can be used to aid with conservation management such as monitoring population sizes over time, monitoring for the presence of an elusive species across a landscape, detecting the occurrence of genetic bottlenecks and inbreeding (Boulanger, Himmer, & Swan, 2004; Fernández, Delibes, & Palomares, 2006; Norén et al., 2016; da Silva & Tolley, 2018). Genetic monitoring can also be used to monitor the population size of species that are rare or difficult to capture by using non-invasive DNA collection methods (Boulanger et al., 2004; Schwartz et al., 2006). For example, Boulanger et al. (2004) collected hair from grizzly bears (*Ursus arctos*) using barbed wire strung across trails; this DNA was then used to make inferences about population trends as a result of changing environmental conditions. Furthermore, genetic monitoring can be used in combination with landscape data to predict the presence of an elusive species. For example, the presence of the Iberian Lynx (*Lynx pardinus*) in Spain, was determined with around 85% accuracy, using DNA samples from faeces (Fernández et al., 2006). Genetic techniques can also be used to manage fragmented populations and to detect bottlenecks (Menotti-Raymond & O'Brien, 1993). For instance, da Silva and Tolley (2018) quantified the genetic diversity of a South African bufonid (*Capensibufo rosei*), a species that has only two known wild populations left. They found evidence that each population underwent a genetic bottleneck, but despite this, the genetic diversity of *C. rosei* was found to be similar to other anurans (da Silva & Tolley, 2018). By recording the genetic diversity annually, the genetic erosion of *C. rosei*

can be monitored in order to inform adaptive management strategies (da Silva & Tolley, 2018). Additionally, genetic monitoring can be used to detect inbreeding (Taylor et al., 2017), often associated with severe genetic bottlenecks (Andersen, Fog, & Damgaard, 2004; Osborne et al., 2016; Maya-García et al., 2017). Norén et al. (2016) monitored a population of the critically endangered artic fox (*Vulpes lagopus*) over a period of nine years. They were able to detect a significant increase in inbreeding over the course of the study, with reduced survival and reproduction in inbred individuals (Norén et al., 2016).

Long term genetic monitoring is also needed to identify any significant trends that differ from normal background environmental variability (Magurran et al., 2010). However, long-term studies are not frequently conducted due to the difficulty in sustaining continued research funding and because the results from such studies may take decades to come to fruition (Strayer et al., 1986; Wandeler, Hoeck, & Keller, 2007). However, a valuable alternative is archived data such as natural history specimens held by museums that have specimens representing a large temporal span, ranging from millions of years ago (e.g. fossils) to the present (Suarez & Tsutsui, 2004; Wandeler et al., 2007). Museum specimens can be used to determine historical genetic variation and this can be compared with current patterns to determine change over time (Cozzolino et al., 2007; Diedericks et al., 2018). Therefore, historical genetic data is a valuable resource for improving our understanding of current patterns of genetic diversity and can also be used to inform management decisions (Wandeler et al., 2007; Bryant et al., 2016). For instance, Bryant et al. (2016) found a 30% decrease in genetic diversity in the Hainan gibbon (*Nomascus hainanus*) compared to historical samples; this can inform management decisions such as the translocation of individuals to form new founder populations.

The Yellow-billed Duck, *Anas undulata*, is widespread and common in south-eastern Africa (Maclean, 1997; South African Bird Atlas Project 2, 2018). These ducks are common residents in South Africa with no regular migrations, showing mostly localised dispersal (≤ 50km) (Brown, Urban, & Newman, 1983; Oatley & Prys-Jones, 1986; Maclean, 1997). The average life expectancy of a Yellow-billed Duck is 3.72 years and they are able to breed as yearlings, however, there is still limited knowledge about the life history traits of Yellow-billed Ducks (Dean & Skead, 1989). Many duck species have been hunted in South Africa for sport and meat over the last century, with Yellow-billed Ducks being a common target (Skead, 1980; Little, Vester, & Crowe, 1995; Maclean, 1997). However, the number of Yellow-billed Ducks that are removed through hunting does not seem to pose a significant threat to the Yellow-billed Duck's population numbers (Dean & Skead, 1989). Another potential threat to the

Yellow-billed Duck population is expanding agriculture that leads to the destruction of natural habitats such as wetlands (Skead, 1980). Additionally, when livestock farming occurs near Yellow-billed Duck habitats, grazing can lead to the reduction of nesting sites and nest building materials (Skead, 1980). Agriculture is expanding in South Africa, potentially increasing the occurrence of these threats (Biggs & Scholes, 2002; Rouget et al., 2003). On the other hand, the expansion of human-made habitats also increases artificial water sources which can be suitable year-round habitats for waterfowl, including Yellow-billed Ducks (Bélanger & Couture, 1988; Traut & Hostetler, 2004; Sebastián-González, Sánchez-Zapata, & Botella, 2010). These habitats are also favoured by invasive birds, such as Mallard Ducks (*Anas platyrhynchos*; Donaldson, Henein, & Runtz, 2007). Invasive Mallard Ducks are known to hybridize with closely related species throughout the world. Consequently, some have argued, based on anecdotal evidence, that one of the main threats to Yellow-billed Ducks in South Africa is hybridization with the invasive Mallard Ducks (Dean, 2000; Owen et al., 2006; Stafford, 2010).

Hybridization between Mallard and Yellow-billed Ducks has been reported, based on observations, across the majority of the provinces of South Africa (Brooke, 1988; Dean, 2000; Roberts, 2003; Joubert, 2009). Invasive Mallard Ducks occur throughout South Africa, which, if claims of hybridization are true, makes them a significant threat to Yellow-billed Ducks (Stafford, 2010; South African Bird Atlas Project 2, 2018). Observations and claims of hybridization are likely correct given that Mallard and Yellow-billed Ducks occupy the same niche, have similar courtship behaviour, calls and resemblance - making them a likely alternative mate choice (Skead, 1980; Roberts, 2003; Joubert, 2009). In addition to the threat from hybridization, Mallards also compete with Yellow-billed Ducks for available habitats and food, dominating ponds, often displacing Yellow-billed Ducks (Banks et al., 2008).

The threats posed by Mallard Ducks to the genetic integrity of Yellow-billed Ducks, call for an urgent need to obtain baseline genetic data for Yellow-billed Ducks in South Africa. Knowledge on population genetic diversity and its structure can aid in management prioritization; i.e. conservation units corresponding to genetically unique populations, or augmentation of genetic diversity by translocations between panmictic populations (Moritz, 1999; Hopken et al., 2015; Weeks et al., 2017). Furthermore, knowledge on historical genetic diversity within the species can help with understanding the impacts of different pressures on Yellow-billed Duck genetic diversity and structure.

In this Chapter I assess the genetic diversity and structure of three contemporary Yellow-billed Duck populations and historical museum samples. I use nuclear microsatellite DNA markers to determine: i) whether the current Yellow-billed Duck populations in South Africa represent one or multiple structured populations ii) the genetic diversity of current Yellow-billed Duck populations in South Africa; and iii) whether genetic diversity and structure of current and historical populations of Yellow-billed Ducks in South Africa differ. My hypotheses are: i) that current populations of Yellow-billed Ducks will have little population structure as migration occurs frequently between populations; ii) that current populations of Yellow-billed Ducks will have a low level of inbreeding and similar levels genetic diversity to other *Anas* species as they are widespread and migration could occur between populations; and iii) historical Yellow-billed Duck samples will have similar genetic diversity to contemporary samples given that there have been no dramatic changes, and therefore bottlenecks, in population numbers.

Materials and Methods

Sampling and DNA extraction

Two-hundred and thirty-four Yellow-billed Duck blood (stored in Queen's buffer) and feather samples, collected in 2013 and 2014, were donated by Chevonne Reynolds from the Percy Fitzpatrick Institute of Ornithology (Supplementary data S1). Two-hundred and two samples were collected from Barberspan in the North West Province and 32 samples were from Strandfontein Sewage Works in the Western Cape. Blood (stored in anticoagulant tubes) and feather samples from five Yellow-billed Duck individuals (assigned to the Yellow-billed Duck genotype class using NEWHYBRIDS (Anderson & Thompson, 2002) (see Chapter Three) were obtained from a Mallard control program conducted by the City of Cape Town (Supplementary data S1). Additionally, Yellow-billed Duck tissue and feather samples were donated by various museums (Table 2a; Supplementary data S1). A 2 × 4 mm piece of tissue was cut using a scalpel blade, using a new blade for each specimen. Samples from outside of South Africa were also included due to the low number of museum samples.

Museum samples and those obtained from the Percy Fitzpatrick institute were stored at room temperature until further use, and the samples from the Mallard control program were stored at -80 °C until further use. DNA was extracted from these samples using the DNeasy Blood and Tissue kit (Qiagen, supplied by Whitehead Scientific, Cape Town, South Africa) following the manufacturer's protocol with slight modifications. For the blood samples, up to 100 µl of blood was used and the incubation time was extended to 1 hour with vortexing every 15 minutes. For the elution step, 50 µl of elution buffer was added. Feather samples were extracted

using a user-developed protocol for feather extraction using the DNeasy Blood and Tissue kit (Qiagen). For this, 2-5 cm was cut from the base of larger feathers and for smaller feathers the entire calamus was included. The base of the feather was crushed using liquid nitrogen before being cut up into small pieces. Scissors used to cut up the feathers were cleaned with 100% ethanol between samples. I added 20 µl 1M DTT solution, 20 µl proteinase K and 300 µl Buffer ATL to the crushed feather, before overnight incubation at 56 °C. The protocol was then continued as per the manufacturer's protocol with the exception of adding 50 µl of elution buffer at the final elution step.

Tissue samples were extracted using the same method as the feather samples but without the addition of DTT and adjusting the first incubation time to 24 hours. To minimize contamination, the first three steps were conducted in a laminar flow cabinet. This precaution was also taken for the museum feather samples. All DNA samples were then quantified using a micro-volume UV-Vis spectrophotometer (Nanodrop, Thermo Fisher Scientific, Waltham, Massachusetts, United States). DNA samples were then frozen at -80 °C until further use.

Table 2a: Samples of Yellow-billed Ducks from museum collections (46 samples in total). Numbers in brackets indicate samples that were collected in South Africa

Name of museum	Samples (42)
Durban Natural Science Museum	25 (22)
Iziko Museum	7 (6)
East London Museum	8 (8)
National Museum, Bloemfontein	4 (4)
McGregor Museum	2 (2)

Microsatellite genotyping

Twenty-eight microsatellite primer pairs previously developed for mallards and other *Anas* species were selected for initial screening for cross-amplification and polymerase chain reaction (PCR) optimization (Supplementary data S2) (Paulus & Tiedemann, 2003; Denk, et al., 2004; Huang et al., 2005; Hsiao et al., 2008). All PCRs were conducted in a MultiGene OptiMax thermal cycler (Labnet International, Edison, New Jersey, USA). Each primer pair was tested for amplification success for Yellow-billed Ducks using gradient PCRs. For PCR

amplification each 10 µl reaction mixture contained 10 ng.µl^{-1} DNA, 0.2 mM deoxynucleoside triphosphates (dNTPs) (Thermo Fisher Scientific, Waltham, Massachusetts, United States), 0.5 µM of each primer, 1 µl of 10x PCR reaction buffer, 1.5 mM $MgCl_2$, 0.2 mg.ml^{-1} Bovine Serum Albumin (BSA; Promega, Madison, Wisconsin, United States), 0.1 U.µl^{-1} Taq DNA Polymerase (Super-Therm JMR-801; Hoffman-La Roche, Basel, Switzerland) and 4.8 µl purified H_2O. The published PCR cycle was used for each primer pair (Paulus & Tiedemann, 2003; Denk, et al., 2004; Huang et al., 2005; Hsiao et al., 2008) but with a gradient annealing temperature between 48 °C and 65 °C to identify the optimal annealing temperature for each locus. Agarose gel electrophoresis using a 3% gel was used to verify amplification.

Sixteen primer pairs that consistently gave good amplification were selected and the forward primer of each labelled fluorescently based on size and annealing temperature to facilitate multiplexing of multiple primers into single reactions. This approach resulted in two multiplexes containing seven and six primer pairs each (Table 2b). Each multiplex was amplified for each individual, with each 15 µl PCR reaction containing 2 ng.µl^{-1} DNA, 1.5 µl of primer mix (concentration of each primer provided in Table 2b), 7.5 µl KAPA2G Fast Multiplex Mix (Kapa Biosystems, supplied by Merck, Cape Town, South Africa) and 4.5 µl purified H_2O. PCR conditions were an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, elongation at 72 °C for 25 s and a final elongation at 72 °C for 1 min. PCR plates with 96 wells each were used, containing 84 unique duck samples, 10 replicate samples and two negative controls. PCR products were run on a 3% agarose gel to verify amplification. Purified PCR fragments were separated at the Central Analytical Facility (Stellenbosch University, Stellenbosch, South Africa) using an ABI PRISM 3100 Genetic analyser (Thermo Fisher Scientific, Waltham, Massachusetts, United States) and sized using the GENE-SCAN -500 (-250) as an internal size standard (Thermo Fisher Scientific, Waltham, Massachusetts, United States). Allele sizes were scored using GeneMarker (Version 2.6.7; SoftGenetics LLC, Pennsylvania, USA). Genotype repeatability was 100% (comparison of 1484 alleles retrieved from 46 repeats). After scoring, primer pair APT016 was removed from multiplex one due to excessive stutter bands and difficulty to score.

Table 2b: Primer multiplexes used for nuclear microsatellite genotyping (N_A: Number of alleles; PIC: Polymorphism information content)

Multiplex	Locus	Size	Label	N_A	PIC	Reference	Concentration for Primer Mix
1	APT016	112-120	FAM	NA	NA	Hsiao et al. 2008	2µM
1	Smo7	184-188	FAM	5	0.645	Paulus & Tiedemann, 2003	2µM
1	CAUD031	112-126	NED	8	0.702	Huang et al. 2005	2µM
1	APT004	294-322	NED	6	0.712	Hsiao et al. 2008	2µM
1	CAUD026	140-150	HEX	9	0.628	Huang et al. 2005	2µM
1	CAUD005	250-284	HEX	10	0.302	Huang et al. 2005	4µM
1	APT015	126-150	PET	8	0.674	Hsiao et al. 2008	2µM
2	APT001	178-206	FAM	1	0	Hsiao et al. 2008	2µM
2	APT014	317-325	FAM	7	0.648	Hsiao et al. 2008	2µM
2	Apl12	112-155	NED	3	0.102	Denk et al. 2004	2µM
2	CAUD004	199-221	NED	7	0.253	Huang et al. 2005	2µM
2	CAUD014	113-117	PET	4	0.177	Huang et al. 2005	2µM
2	CAUD035	223-237	PET	4	0.359	Huang et al. 2005	2µM

Genetic diversity

Samples that had 50% or more missing data were removed from all further analyses. Samples that were classified as hybrids by NEWHYBRIDS (Anderson & Thompson, 2002; see Chapter Three) were removed from the data set. The subsequent data set consisted of 242 samples. The data was checked for null alleles and scoring errors using Micro-Checker version 2.2.3 (Van Oosterhout et al., 2004). Null alleles were detected and consequently the software FreeNA (Chapuis & Estoup, 2007) was used to calculate corrected (i.e., without null alleles using the ENA method described in Chapuis and Estoup (2007)) and uncorrected estimates of pairwise F_{ST} values (Weir, 1996). To test for a significant difference between corrected and uncorrected

pairwise F_{ST} values an analysis of variance (ANOVA) was conducted in the R statistical environment version 3.5.1 (R Development Core Team, 2017). Genotype data was tested for allele frequency departures from Hardy-Weinberg equilibrium (HWE) expectations using the package pegas (version 0.11, Paradis, 2010) in R (R Development Core Team, 2017).

Allelic richness (A_R), inbreeding coefficients (F_{IS}) and observed and expected heterozygosity (H_O and H_E) were calculated for each population using the diveRsity R package (version 1.9.90, Keenan et al., 2013; R Development Core Team, 2017). Rarefaction was applied for A_R calculations due to the large differences in population sizes. Private allelic richness (PA_R) was calculated per population with rarefaction using the program ADZE (Allelic Diversity Analyzer; version 1.0; Szpiech, Jakobsson, & Rosenberg, 2008). Five individual heterozygosity measures were calculated (proportion of heterozygous loci in an individual (*PHt*); standardised heterozygosity based on mean expected heterozygosity (*Hs_exp*); standardised heterozygosity based on mean observed heterozygosity (*Hs_obs*); internal relatedness (*IR*); and homozygosity by locus (*HL*)) using the function GENHET version 3.1 in R (Coulon, 2010; R Development Core Team, 2017). To test if there was a significant difference in individual diversity measures (PHt, Hs_exp, Hs_obs, IR and HL) across populations, I used ANOVA and Kruskal-Wallis tests in R (R Development Core Team, 2017).

Genetic structure

I used STRUCTURE version 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) to determine the number of genetic clusters (*K*). Assignment values (q_{ik}) in STRUCTURE are calculated as the proportion of an individual's genotype that belongs to each genetic cluster. Values of *K* from 1 to 14 were tested as samples represented 14 different locations. I used an admixture model with correlated allele frequencies, 100,000 burn-in iterations, 1,000,000 Markov Chain Monte Carlo repetitions, and 20 iterations per run. STRUCTURE HARVESTER (version 0.6.94, Earl & vonHoldt, 2012) was used to determine to optimum number of *K* using the delta K method of Evanno et al. (2005). DISTRUCT (version 1.1, Rosenberg, 2004) was used to graphically visualise the STRUCTURE results. A Principal Components Analysis (PCA) done using the ade4 and adegraphics R packages (Thioulouse et al., 1997; Siberchicot et al., 2017; R Development Core Team, 2017) was used as an additional means to visualise population genetic structure between Yellow-billed Duck populations.

Population pairwise F_{ST} values were calculated using GenAlEx (version 6.5, Peakall & Smouse, 2006). To assess the genetic variation between and among Yellow-billed Duck

populations, an analysis of molecular variance (AMOVA) was performed using GenAlEx (version 6.5, Peakall & Smouse, 2006).

I estimated the rate of gene flow between Barberspan and Cape Town (Strandfontein and Marina Da Gama) populations using BayesAss (version 3.0.4, Wilson & Rannala, 2003). For this analysis I used the mixing parameters dM = 0.05, dA = 0.2, dF = 0.2. The number of MCMC iterations was 1,000,000 with a burn-in of 10,000 and a sampling interval of 1000.

Results

Genetic diversity

The final data set consisted of 242 samples consisting of four populations (Barberspan (n = 195); Strandfontein (n = 25); Marina Da Gama (n = 5) and museum samples (n = 17)). One locus (APT001) was removed as it was monomorphic for all Yellow-billed Duck samples genotyped. Consequently, the final data set consisted of 11 loci. Four loci showed significant departures from HWE for at least three of the populations included here. This is likely because the populations do not meet the assumptions of no migration and gene flow. No significant difference was detected between pairwise F_{ST} values corrected for null alleles and uncorrected pairwise F_{ST} values ($F_{1,10}$ = 1.575, p = 0.238) and a low average null allele frequency was detected (0.047, standard deviation = 0.069). Therefore, all further analyses were conducted using uncorrected data. Genetic diversity measures were moderate across populations and there was only a small difference between observed and expected heterozygosity (H_O: 0.39 - 0.44; H_E: 0.39 - 0.49; Table 2c). Populations thus showed low levels of inbreeding (F_{IS}: 0.01 - 0.18; Table 2c). On the other hand, allelic richness and private allelic richness measures were low (A_R: 2.39 - 3.24; PA_R; 0.21 - 0.54; Table 2c). Individual diversity measures were not significantly different among populations (Table 2d) and were similar to the population genetic parameters. The proportion of heterozygous loci was also moderate across populations (*PHt*: 0.37 - 0.42; Table 2d). The other heterozygosity measures were a bit higher (*Hs_exp*: 0.81 - 0.89; *Hs_obs*: 0.97 - 1.06; Table 2d) but each marker is given the same weight in the calculation. Internal relatedness was low (*IR*: 0.10 - 0.17; Table 2d), similar to the levels of inbreeding detected when using population genetic parameters.

Table 2c: Population genetic parameters (mean across loci) for the four sampled populations of Yellow-billed Ducks are provided: number of samples (N)*; allelic richness* (A_R)*; observed* (H_O) *and expected heterozygosity* (H_E)*; inbreeding coefficients* (F_{IS})*; and private allelic richness* (PA_R)*. Standard deviations are indicated in brackets*

Population	N	A_R	H_O	H_E	F_{IS}	PA_R
Barberspan	195	2.81 (0.94)	0.41 (0.24)	0.49 (0.25)	0.18 (0.25)	0.31 (0.16)
Strandfontein	25	2.81 (1.11)	0.39 (0.29)	0.46 (0.27)	0.18 (0.26)	0.38 (0.19)
Marina Da Gama	5	3.24 (0.99)	0.44 (0.19)	0.47 (0.19)	0.01 (0.26)	0.54 (0.51)
Museum samples	17	2.39 (0.75)	0.41 (0.34)	0.39 (0.23)	0.08 (0.53)	0.21 (0.14)

Table 2d: Population genetic parameters (mean across individuals) for the four sampled populations of Yellow-billed Ducks are provided: number of samples (N)*; proportion of heterozygous loci in an individual* (*PHt*)*; standardised heterozygosity based on mean expected heterozygosity* (*Hs_exp*)*; standardised heterozygosity based on mean observed heterozygosity* (*Hs_obs*)*; internal relatedness* (*IR*)*; and homozygosity by locus* (*HL*)*. Standard deviations are indicated in brackets.*

Population	N	*PHt*	*Hs_exp*	*Hs_obs*	*IR*	*HL*
Barberspan	195	0.40 (0.15)	0.85 (0.31)	1.01 (0.37)	0.16 (0.28)	0.49 (0.18)
Strandfontein	25	0.37 (0.12)	0.81 (0.27)	0.98 (0.34)	0.17 (0.27)	0.51 (0.14)
Marina Da Gama	5	0.42 (0.13)	0.89 (0.27)	1.06 (0.32)	0.17 (0.19)	0.51 (0.17)
Museum samples	17	0.37 (0.12)	0.85 (0.25)	0.97 (0.29)	0.10 (0.25)	0.48 (0.14)
P value		0.81	0.97	0.70	0.45	0.91

Genetic structure

Two genetic clusters (K = 2) was determined as the optimal number of clusters by STRUCTURE HARVESTER (Supplementary data Figure S1 and Figure S2) but when examining the STRUCTURE plot most individuals were assigned with almost equal probability to both clusters (Figure 2a). Additionally, when examining the STRUCTURE plot for higher values of K there was almost equal assignment to each genetic cluster (Figure 2a), indicating that there is likely to be no observable genetic structure between the populations that I tested. Pairwise F_{ST} values (F_{ST} < 0.2; Table 2e) and the PCoA supported this apparent lack of population genetic structure (Figure 2b). The AMOVA results showed low (4.5%) but significant (P = 0.001) genetic differentiation between populations (Table 2f).

*Table 2e: Pairwise F_{ST} values calculated according to Weir (1996) for each Yellow-billed Duck population (1 = Barberspan, 2 = Strandfontein, 3 = Marina Da Gama, 4 = museum samples). P values are included in brackets and significant values (P < 0.05) are indicated with a *.*

Population	1	2	3
2	0.007*(0.022)		
3	0.015(0.131)	0.022(0.064)	
4	0.095*(0.001)	0.109*(0.001)	0.094*(0.001)

Gene flow from Barberspan into Cape Town populations was estimated to be 0.3137 migrants per generation (95% credible interval 0.2802-0.3472), while gene flow from Cape Town populations to Barberspan was estimated to be 0.0087 migrants per generation (95% credible interval -0.004-0.0214).

Table 2f: Analysis of Molecular Variance for the four Yellow-billed Duck populations

Source of variation	df	Sum of squares	Variance	Percentage variation (%)
Among populations	3	34.4	0.139	4.5
Among individuals	238	943.6	1.000	32.2
Within Individuals	242	475.5	1.965	63.3

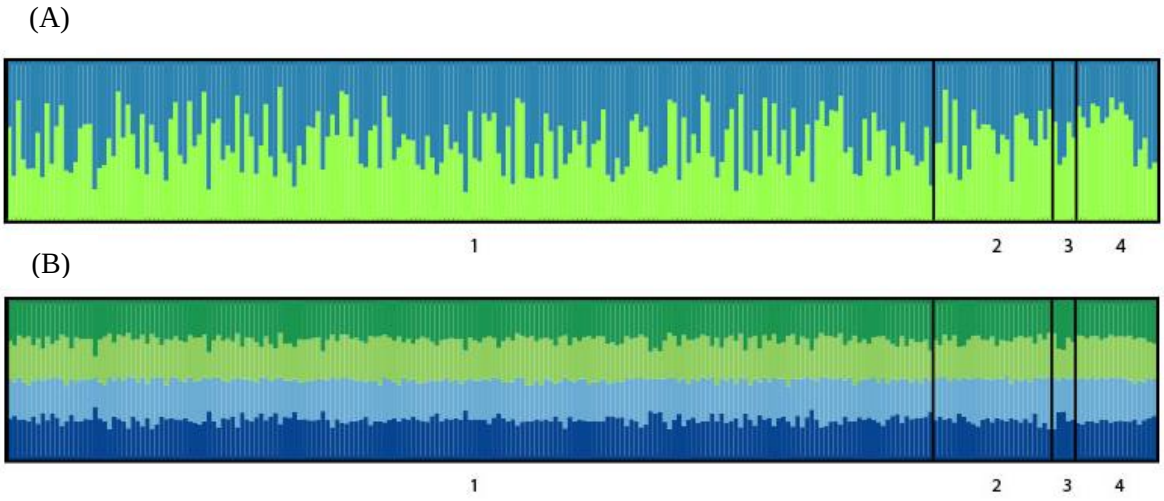

Figure 2a, Structure bar plots where each bar represents an individual and the colour of the bar indicates the proportion of assignment to each genetic cluster. Individuals are organised by population: Barberspan (1), Strandfontein (2), Marina Da Gama (3) and museum samples (4). (A) The top plot shows the assignment values (q_{ik}) when there are two genetic clusters (K = 2). (B) The bottom plot shows the assignment values when there are four genetic clusters (K = 4), this indicates that there is likely no observable genetic structure between populations as they have almost equal assignment to each genetic cluster

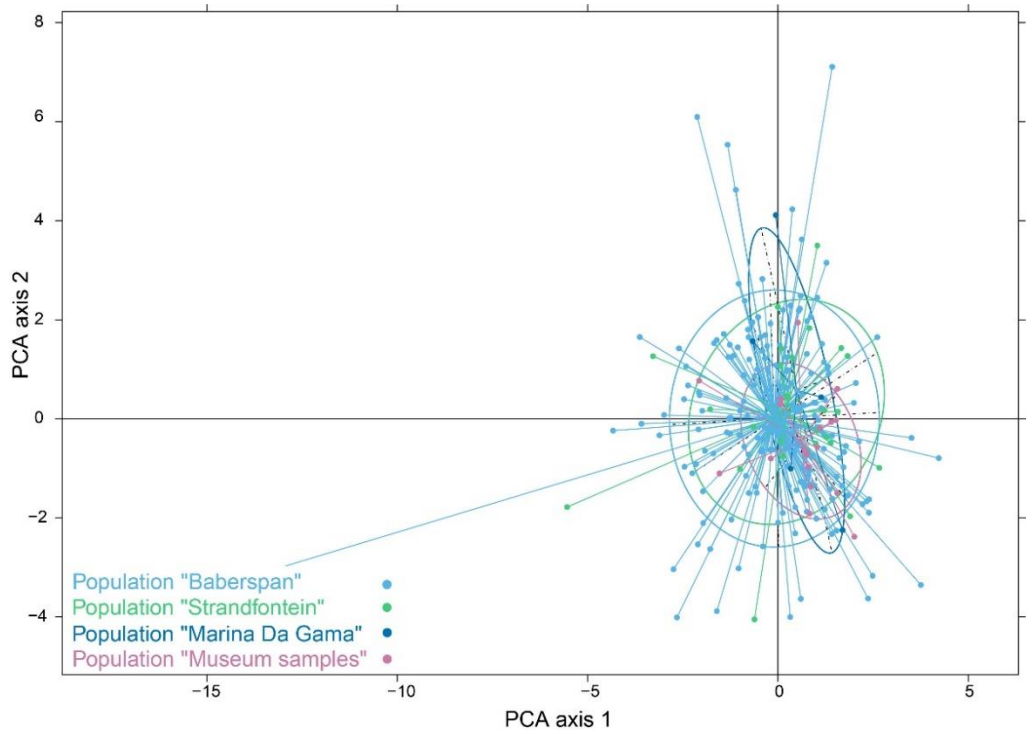

Figure 2b, Scatter plot of principal components analysis displaying the difference in genetic relationships between the four Yellow-billed Duck populations (PCA axis 1 = 4.4%; PCA axis 2 = 4.0%)

Discussion

This study supported my hypothesis of no population genetic structure between the four Yellow-billed Duck populations included here. Additionally, similar genetic diversity measures were found among all four populations indicating that there is sufficient gene flow to maintain similar levels of genetic diversity within populations, with random breeding (i.e. no inbreeding). I also found no difference in population genetic diversity and structure between historical (museum) and contemporary samples, indicating that there has been no major genetic changes, e.g. bottlenecks, in the South African Yellow-billed Duck population, at least over the last 60 years.

Implications of panmixia for Yellow-billed Duck conservation in South Africa

The lack of population structure, even between very widely distributed Yellow-billed Duck populations, most likely reflects frequent long-distance dispersal of the species. This causes sufficient connectivity between widespread populations to prevent the emergence of strong spatial genetic structure. Other duck species (the Mottled Duck (*Anas fulvigula*), the White-headed Duck (*Oxyura leucocephala*) and the Mallard Duck) show a similar lack of genetic structure on a local scale (Williams, Brust, & Rhodes Jr., 2002; Muñoz-Fuentes et al., 2007) but much more prominent population genetic structure over large geographic distances (McCracken, Johnson, & Sheldon, 2001; Kulikova et al., 2005). These strong genetic structures are often driven by geographic boundaries and a lack of gene flow, for instance in the case of the Mottled Duck populations in Texas and Florida (McCracken et al., 2001) and the Mallard Duck which has strong genetic structure between the Old World and the New World (Kulikova et al., 2005). The panmictic population of Yellow-billed Ducks can be interpreted in two ways. Firstly, the lack of population structure indicates that management of Yellow-billed Ducks in South Africa must consider individual 'populations' at a very large, and likely unrealistic, scale. This conservation unit may even extend northwards as some museum samples from outside of South Africa (Zimbabwe) did not differ significantly in genetic diversity or their relatedness to South African birds, suggesting that individuals from that area could also be part of the same conservation unit. The evidence of panmixia and lack of genetic structure across South Africa indicates that there is no evidence of Yellow-billed Duck populations with distinct genetic diversity that needs to be conserved. This means that the Yellow-billed Duck does not need to be a conservation priority, particularly as they are widespread and listed as Least Concern (The IUCN Red List, 2016; South African Bird Atlas Project 2, 2018).

My findings also point to another important conservation issue. Given evidence for hybridization between Yellow-billed and Mallard Ducks (see Chapter Three), frequent long-distance dispersal between far-off Yellow-billed Duck populations implies that hybrid or introgressed genotypes, may reach other far-off Yellow-billed Duck populations, even when they are not in direct contact with Mallard Ducks. This can possibly lead to an intriguing phenomenon where hybridization spreads through the environment in the absence of one of the parental species.

Although my study supports panmixia for Yellow-billed Duck populations in South Africa there are limitations that need to be considered given that only a few populations were sampled. The markers that I used could not have been informative enough to detect genetic structure between the populations (Selkoe & Toonen, 2006). Also, further sampling throughout the Yellow-billed Duck distribution would be necessary to determine if the Yellow-billed Duck population is truly panmictic throughout its distribution.

Genetic diversity in contemporary populations

The amount of genetic diversity found in contemporary Yellow-billed Duck populations is similar to the genetic diversity typical of other duck species. Observed and expected heterozygosities were similar to other microsatellite studies conducted on other ducks (Williams, et al., 2005; Khan Ahmadi et al., 2007; Muñoz-Fuentes et al., 2007; Fowler, Eadie, & Engilis, 2009; Wu et al., 2009). I also found no evidence for excessive inbreeding in the Barberspan and Cape Town populations, indicating that mating is random. This may, in part, be facilitated by frequent long-distance dispersal that homogenises genetic diversity between Yellow-billed Duck populations and reduces inbreeding (Lacy, 1987; Simberloff, 1988; Amos & Harwood, 1998). On the other hand, the mean allelic richness for each population was lower on average than other comparable population genetic studies on ducks (Muñoz-Fuentes et al., 2007; Fowler et al., 2009). Large populations are also more likely to have a higher genetic diversity as the impacts of genetic drift are expected to impact genetic diversity less compared to small population sizes (Lacy, 1987; Frankham, 1996).

Comparison of historical (museum) and contemporary Yellow-billed Duck populations

Genetic diversity measures were similar between contemporary and historical Yellow-billed Duck groups. Drastic changes in demographic attributes, e.g. population contractions or expansions, often manifest in genetic diversity contained in populations (Keller et al., 2010; Xenikoudakis et al., 2015; da Silva & Tolley, 2018). For example, a rapid population decline

in Yellow-billed Ducks would have translated into lowered heterozygosity and higher inbreeding in contemporary vs historical individuals. The lack of genetic differentiation between contemporary and historical samples suggests that the Yellow-billed Duck population has not experienced significant demographic changes over the last 60 years. This lack of large demographic changes is likely due to their large range size and abundance, meaning that any localised changes in population size through hunting or habitat destruction, are unlikely to impact their overall population numbers and consequently genetic diversity. Additionally, the lack of change in genetic diversity could also indicate that any recent changes like increasing urbanisation are not impacting negatively on the Yellow-billed Duck population. In fact, Yellow-billed Ducks are able to utilise artificial water sources like sewage works as year-round habitats (Bélanger & Couture, 1988; Traut & Hostetler, 2004; Sebastián-González et al., 2010), and so, are probably able to do well even with increasing urbanisation.

Although I did not detect any major genetic changes between historical and contemporary populations there are several limitations worth considering. Firstly, any localised demographic changes would not have affected overall population numbers and therefore these changes could not be detected in this study. Secondly, no demographic analyses were conducted and therefore these analyses would be needed to confirm the lack of significant demographic changes over the last 60 years. Lastly, the changes could have been left undetected because the markers that I used were not informative enough (Selkoe & Toonen, 2006) and because of the small number of museum samples.

Conclusion

This current populations in Cape Town and Barberspan (and likely the rest of South Africa) were found to represent a panmictic population. This has two implications for the conservation of the Yellow-billed Duck. Firstly, this means that resources should not be focused on conserving any distinct genetic diversity in local populations. Secondly, in terms of the main threat to the species: hybridization with the Mallard Duck; it also means that there is potential for hybrid genes to spread between Yellow-billed Duck populations. Additionally, genetic diversity and structure were found to have not changed significantly over time, suggesting that the Yellow-billed Duck population has not had any major changes in population numbers and appears to be ‘healthy’. This could indicate that Yellow-billed Ducks are not, at least up until now, negatively impacted by threats such as urbanisation.

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Chapter Three Abstract

Hybridization with invasive alien species can negatively impact native congeners through genetic introgression and pollution. This can lead to reduced fitness of the native population through the loss of unique genotypes and/or co-adapted gene complexes.

Invasive Mallard Ducks *(Anas platyrhynchos)* hybridize with several closely related native species within the genus *Anas*, including the native South African Yellow-billed Duck (*Anas undulata*). These two species are able to produce fertile offspring and therefore Mallards are a potential threat to the long-term genetic integrity of this native duck. However, there has been a lot of public opposition to the control of Mallard Ducks in South Africa, partly due toa lack of awareness of the potential threat that Mallard Ducks pose to the Yellow-billed Duck. Despite the threat from hybridization, the perceived hybridization between the two species remains based on observational and anecdotal evidence, with no work having been done on the genetic extent of hybridization between the two species.

Consequently, I use microsatellite genotyping and sequencing of a mitochondrial gene region to investigate hybridization between invasive Mallard Ducks and native Yellow-billed Ducks in South Africa. There is evidence of hybridization between the two species, but most backcrossing and introgression is occurring into the Mallard Duck population. This means that for now, the Yellow-billed Duck population is largely unpolluted by Mallard Duck genes. However, introgression could become more extensive in the future. I also found evidence of sex-biased mating, with most mating occurring between Mallard Duck hens and Yellow-billed Duck drakes. These findings indicate that Mallard Duck hens should be prioritised for removal and that it is advisable to remove Mallard Ducks and their hybrids while such interventions will have a high chance of success. As introgression is not yet extensive, there is still a good chance of protecting the genetic integrity of the Yellow-billed Duck. This evidence can be used to gain public support for control of Mallard Ducks in South Africa.

Chapter Three

The incidence and extent of hybridization and introgression between invasive Mallard Ducks (*Anas platyrhynchos*) and native Yellow-billed Ducks (*Anas undulata*) in South Africa

Introduction

Invasive non-native species are recognised as one of the most significant threats to native biodiversity globally (Clavero & Garccıa-Berthou, 2005; McGeoch et al., 2010). There are a wide range of negative impacts caused by invasive species including competition, predation and hybridization (Blackburn et al., 2014). However, some of these impacts are generally under investigated; one example is the negative impact of hybridization (Rhymer & Simberloff, 1996). Hybridization is one of the top ten negative impacts of invasive species on native biodiversity and has been shown to contribute to population decline of native species, in some instances even contributing to their extinction (Rhymer & Simberloff, 1996; Wolf, Takebayashi, & Rieseberg, 2001; Blackburn et al., 2014; Todesco et al., 2016). When hybrid offspring are fertile, hybridization between native and non-native species can result in successive backcrossing and, ultimately, genetic introgression (Rhymer & Simberloff, 1996; Schulte, Veith, & Hochkirch, 2012). Genetic introgression can cause the loss of genetic uniqueness and of native and locally adapted genotypes, which can reduce the fitness of the native population (Allendorf et al., 2001; Olden et al., 2004; Schulte et al., 2012). If genetic introgression is extensive it can eventually result in the extinction of the native species (Rhymer & Simberloff, 1996; Wolf et al., 2001; Todesco et al., 2016).

A poster child for the impacts of hybridization by invasive species is the Mallard Duck, *Anas platyrhynchos*. This species is known to hybridize with several closely related native species across the world, within its own genus, including: the Florida Mottled Duck (*A. fulvigula*); the American Black Duck (*A. rubripes*); the New Zealand Grey Duck (*A. superciliosa superciliosa*) and the Hawaiian Duck (*A. wyvilliana*) (Rhymer, Williams, & Braun, 1994; Mank, Carlson, & Brittingham, 2004; Fowler, Eadie, & Engilis, 2009). Many of these hybrids are fertile, which threatens the genetic integrity of native ducks through genetic introgression (Johnsgard, 1960; Rhymer & Simberloff, 1996). Additionally, Seymour (1990) and D'Eon, Seymour and Boer (1994) observed aggressive mating behaviour in Mallard Ducks; this aggressive mating behaviour could contribute to their ability to outcompete native drakes for available mating opportunities with resident native species (Brodsky & Weatherhead, 1984).

Mallard Ducks have a large native Northern Hemisphere range, mainly across Europe and North America (The IUCN Red List, 2017). Their widespread distribution is mainly due to artificial management of their populations because of their popularity as game and ornamental birds (Long, 1981; Rhymer, 2006; Champagnon et al, 2009; ČíŽková et al., 2012). In Europe, large scale releases of Mallard Ducks began in the 1970s, with releases of more than one million captive bred individuals a year still occurring (Champagnon et al., 2009). In North America, Mallard Duck numbers have also been artificially augmented through the continual release of large numbers of individuals for game hunting (Heusmann, 1991). The large number of Mallard Ducks released has, in part, facilitated hybridization between Mallard Ducks and the American Black Duck (Heusmann, 1991; Mank et al., 2004). Prior to 1900 these two species were allopatric but, deforestation and habitat change has led to a decline in habitats suitable for black ducks (Brodsky & Weatherhead, 1984; Mank et al., 2004). This loss of habitat, combined with large Mallard Duck releases, and an increase in Mallard Ducks in these habitats, has led to contact between the two species and subsequent hybridization (Brodsky & Weatherhead, 1984; Heusmann, 1991). Brodsky and Weatherhead (1984) found that, although Mallard Duck drakes prefer Mallard Duck hens as mates, they outcompete black duck drakes for black duck hens after all Mallard Duck hens have been paired up. Mank et al. (2004) found less genetic diversification between modern black duck and Mallard Duck lineages, compared to historical museum samples, indicating that recent introgression has occurred, reducing the genetic uniqueness of the American Black Duck. It is thus probable that the combination of hybridization and loss of habitat will lead to the extinction of the "pure" American Black Duck genetic lineage in the future (Brodsky & Weatherhead, 1984).

The Mallard Duck also threatens the New Zealand Grey Duck through hybridization. Mallard Ducks were introduced into New Zealand in the mid-1800s and again in the 1930s (Rhymer et al., 1994). As in the case of the North American Black Duck, habitat change has facilitated hybridization between grey ducks and Mallard Ducks, leading to a decline in the grey duck population (Gillespie, 1985; Rhymer et al., 1994). Gillespie (1985) found increased numbers of hybrids to be correlated with a corresponding decrease in genetically pure grey ducks. Hybrid individuals can now be found across New Zealand with introgression occurring into both Mallard Duck and grey duck populations, which may eventually lead to the extinction of grey ducks (Rhymer et al., 1994).

Hybridization between Mallard Ducks and native duck species is often sex-biased, this can go two ways; in the first case most mating occurs between Mallard Duck drakes and native duck

hens. However, in the second case, sex-biased mating can go the other way where most mating occurs between Mallard Duck hens and native males. This is the case with hybridization between the Mallard Duck and the endangered Hawaiian Duck. Fowler et al. (2009) found evidence of sex-biased mating, with most hybrids likely resulting from Mallard Duck hen and Hawaiian Duck drake crosses. They suggest that it is because of the Hawaiian Duck's active courtship behaviour and Mallard Duck hen's relaxed mating preference, as they originated from domestic stock (Fowler et al., 2009). In Hawaii pure and isolated Hawaiian Duck populations can still be found on some islands such as Kaua'i and Ni'ihau (Engilis, Uyehara, & Giffin, 2002; Fowler et al., 2009). However, preventing hybridization from occurring on these islands is difficult as hybrid swarms on neighbouring islands are likely to disperse naturally between islands. A hybrid individual has already been found on Kaua'i (Engilis et al., 2002; Fowler et al., 2009).

Mallard Ducks were introduced into southern Africa in the 1940s (Liversidge, 1985). The history of Mallard Duck introductions (i.e. timing, propagule pressure) into South Africa is largely unknown (Liversidge, 1985; Dean, 2000). Currently in South Africa, naturalized populations of Mallard Ducks are widespread, likely representing the early stages of a large scale invasion (Stafford, 2010; South African Bird Atlas Project 2, 2018a). Mallard Ducks are mainly found in the southern parts of the Western Cape Province and in the Gauteng Province, but have also been recorded in all other provinces of the country (Stafford, 2010; South African Bird Atlas Project 2, 2018a). Secondary introductions continue to occur from within South Africa as Mallard Ducks are commonly used as an ornamental species to stock domestic ponds (Brooke, 1988; Joubert, 2009). Mallard Ducks are currently listed as a category two invasive species according to the National Environmental Management Biodiversity Act (Act 10 of 2004: Alien and Invasive Species Regulations), which means that a permit is needed to keep them (Government of the Republic of South Africa, 2016). Furthermore, these regulations stipulate that Mallard Ducks must be controlled in areas not currently falling under permits by the person in possession of the invasive species or as part of the official Invasive Species Management Programme (Government of the Republic of South Africa, 2014).

Unsurprisingly, observational data suggests that Mallard Ducks are able to hybridize with South African *Anas* species, notably Cape Shovelers (*A. smithii*), Cape Teal (*A. capensis*) and African Black Ducks (*A. sparsa*), and especially, Yellow-billed Ducks (*A. undulata*). For the latter, hybrids have been reported in the Eastern Cape, Free State, Gauteng, KwaZulu-Natal and Western Cape Provinces (Brooke, 1988; Dean, 2000; Roberts, 2003; Joubert, 2009;

Stafford, 2010). There have also been observations of mating between Mallard Duck drakes and Yellow-billed Duck hens. Mallard Ducks are a likely alternative mate choice for Yellow-billed Ducks due to the similar calls and resemblance of the hens of the two species (Skead, 1980; Joubert, 2009). These two duck species are able to produce fertile offspring, and therefore Mallard Ducks pose a significant threat to the genetic integrity of Yellow-billed Ducks (Johnsgard, 1960; Rhymer & Simberloff, 1996; Roberts, 2003; Stafford, 2010). Additionally, Mallard Ducks not only pose a threat through hybridization, but they are also likely to compete with Yellow-billed Ducks for habitat and food. They often dominate ponds, resulting in the displacement of Yellow-billed Ducks (Banks et al., 2008).

Given the perceived impacts of Mallard Ducks on native ducks and in accordance with South African law, a control program has been introduced in Cape Town. However, there has been strong public opposition to the program (Banks et al., 2008; Stafford, 2010) as many residents enjoy feeding Mallard Ducks and consider them as pets. Additionally, it is also believed that the public's lack of awareness of the threat that Mallard Ducks pose to native ducks, is one of the main reasons that the public is against Mallard Duck control (Stafford, 2010). The public see Mallard Ducks as visually appealing and harmless and many perceive their removal to be inhumane and cruel. Residents have even threatened public officials who were trying to remove Mallard Ducks. This opposition makes it challenging to execute the control program and can also slow the control program. The lack of scientific evidence and the strong public opposition calls for an urgent and scientifically-based assessment of hybridization between Mallard Ducks and native Yellow-billed Ducks. In this chapter, I assess the incidence of hybridization between Mallard Duck and Yellow-billed Duck in South Africa with a focus on populations in Cape Town. I use microsatellite markers and mitochondrial DNA to i) identify putative hybrid individuals and determine if introgression is occurring, and ii) to determine if there are sex-biased mating preferences. My hypotheses are i) that there is evidence of extensive hybridization and introgression between Mallard and Yellow-billed Ducks with more introgression occurring in the Yellow-billed Duck population, and ii) that there is sex-biased mating occurring with more hybridization between Mallard Duck drakes and Yellow-billed Duck hens. The results of this study could help to inform control efforts andeducate the public, and thereby hopefully persuade them in favour of any management options.

Materials and Methods

Sampling and DNA extraction

Blood and feather samples from 121 individuals (Mallard Ducks and putative Mallard Duck × Yellow-billed Duck hybrids) were obtained from a Mallard Duck control program conducted by the City of Cape Town (Supplementary data S3). The research project was approved by the Stellenbosch University Research Ethics Committee (Application number: SU-ACUD17-00042). Ducks were sedated using alpha-chloralose placed in bread and were then removed to a suitable onsite location where blood samples and euthanasia could be conducted. A qualified veterinarian from the City of Cape Town was responsible for blood collections and euthanisation of all ducks. For each duck, mass was measured, measurements (bill, head, tarsal wing, wingspan, total body length, tarsus) were taken, and a photograph was taken for classification (i.e. Mallard Duck or putative hybrid). At least four flight feathers were also removed for additional DNA material. The carcasses were then removed by the City of Cape Town and collected by State veterinarian for avian flu testing after which they were incinerated.

The Yellow-billed Duck blood and feather samples (n = 234; Barberspan (n = 202 and Strandfontein (n = 32)) from Chapter Two were used, that were donated by Chevonne Reynolds (Supplementary data S3). Additionally, 51 tissue and feather samples of Mallard Ducks, Yellow-billed Ducks, and putative hybrids were donated by various museums (Supplementary data S3): each piece of tissue measured 2 × 4 mm and was cut using a new scalpel blade for each specimen.

The Yellow-billed Duck blood and feather samples and the museum samples were stored at room temperature until further use, as they had been previously. All blood and feather samples from the Mallard Control Program were stored at -80 °C until further use. The DNeasy Blood and Tissue kit was used to extract DNA from the samples (Qiagen, supplied by Whitehead Scientific, Cape Town, South Africa) following the manufacturer's protocol with slight alterations. When extracting the blood samples, , up to 100 µl of untreated blood, blood stored in anticoagulant tubes, or blood stored in Queen's buffer was used, and I extended the incubation time to 1 hour with vortexing every 15 minutes. I adjusted the amount of elution buffer added during the elution step to 50 µl. When extracting feather samples, I used a user-developed protocol for feather extraction and the DNeasy Blood and Tissue kit (Qiagen). For large feathers 2-5 cm was cut from the base, whereas the entire calamus was included for smaller feathers. Liquid nitrogen was used to crush the base of the feather and then it was cut up into small pieces. The scissors were cleaned with 100% ethanol between samples. Then, 20

µl 1M DTT solution, 20 µl proteinase K and 300 µl Buffer ATL were added to the crushed feather, and it was incubated overnight at 56 °C. The rest of the protocol was then continued as per the manufacturer protocol with the exception of adjusting the elution buffer to 50 µl at the elution step. Seven feather samples were extracted for sequencing using a DNA salt extraction protocol (MacManes, 2013) because the samples did not yield high enough DNA concentrations for sequencing when using the protocol described above.

Tissue samples were extracted using the same method as the feather samples however, DTT solution was not added and the first incubation time was adjusted to 24 hours. Additionally, the first three steps were conducted in a laminar flow cabinet to minimize contamination. This precaution was also conducted when extracting the museum feather samples and the first incubation time was also adjusted to 24 hours, as with the other museum samples. A micro-volume UV-Vis spectrophotometer (Nanodrop, Thermo Fisher Scientific, Waltham, Massachusetts, United States) was used to quantify all DNA samples. DNA samples were then frozen at -80 °C until further use.

Microsatellite genotyping and DNA sequencing

PCR optimization of 28 microsatellite primer pairs (previously developed for Mallard Ducks and other *Anas* species) was conducted (Supplementary data S2, Paulus & Tiedemann, 2003; Denk, et al., 2004; Huang et al., 2005; Hsiao et al., 2008). A MultiGene OptiMax thermal cycler (Labnet International, Edison, New Jersey, USA) was used to conduct all PCRs. Gradients PCRs were used to test each primer pair for amplification success for both Mallard and Yellow-billed Ducks. Each 10 µl PCR reaction mixture contained 10 ng.µl^{-1} DNA, 0.2 mM deoxynucleoside triphosphates (dNTPs) (Thermo Fisher Scientific, Waltham, Massachusetts, United States), 0.5 µM of each primer, 1 µl of 10 × PCR reaction buffer, 1.5 mM $MgCl_2$, 0.2 mg.ml^{-1} Bovine Serum Albumin (BSA; Promega, Madison, Wisconsin, United States), 0.1 U.µl^{-1} Taq DNA Polymerase (Super-Therm JMR-801; Hoffman-La Roche, Basel, Switzerland) and 4.8 µl purified H_2O. I used the published cycle for each primer pair (Paulus & Tiedemann, 2003; Denk, et al., 2004; Huang et al., 2005; Hsiao et al., 2008) and a gradient annealing temperature between 48 °C and 65 °C was used to find optimal annealing temperatures for each species × locus combination. To verify amplification I conducted agarose gel electrophoresis using a 3% gel.

I selected 16 primer pairs that consistently gave good amplification in both species and fluorescently labelled the forward primer of each pair based on size and annealing temperature

to facilitate multiplexing of primers. Labelled primers were subsequently optimized into two multiplexes containing seven and six primer pairs each (Table 3a). Amplification of each multiplex was completed for each individual, with each 15 µl PCR reaction containing 2 ng.µl^{-1} of DNA, 1.5 µl of primer mix (concentration of each primer provided in Table 3a), 7.5 µl KAPA2G Fast Multiplex Mix (Kapa Biosystems, Cape Town, South Africa) and 4.5 µl purified H_2O. The following PCR conditions were used: an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, elongation at 72 °C for 25 s and a final elongation at 72 °C for 1 min. PCR plates with 96 wells each were used, which contained 84 unique duck samples, 10 replicate samples, and two negative controls. Amplification was verified using a 3% agarose gel. Purified PCR fragments were separated at the Central Analytical Facility (Stellenbosch University, Stellenbosch, South Africa) using GENE-SCAN -500 (-250) as internal size standard (Thermo Fisher Scientific, Waltham, Massachusetts, United States) on an ABI PRISM 3100 Genetic analyser (Thermo Fisher Scientific, Waltham, Massachusetts, United States) . GeneMarker (Version 2.6.7; SoftGenetics LLC, Pennsylvania, USA) was used to score allele sizes . Genotype repeatability was 100% (comparison of 1484 alleles retrieved from 46 repeats). After scoring, primer pair APT016 was removed from multiplex one due to difficulty to score and excessive stutter bands. This resulted in 12 loci being included in the final data set.

Table 3a: Primer multiplexes used for nuclear microsatellite genotyping (N_A: Number of alleles; PIC: Polymorphism information content)

Multiplex	Locus	Size	Label	N_A	*PIC*	Reference	Concentration for Primer Mix
1	APT016	112-120	FAM	NA	NA	Hsiao et al. 2008	2µM
1	Smo7	184-188	FAM	7	0.715	Paulus & Tiedemann, 2003	2µM
1	CAUD031	112-126	NED	11	0.816	Huang et al. 2005	2µM
1	APT004	294-322	NED	7	0.702	Hsiao et al. 2008	2µM
1	CAUD026	140-150	HEX	11	0.670	Huang et al. 2005	2µM
1	CAUD005	250-284	HEX	13	0.421	Huang et al. 2005	4µM
1	APT015	126-150	PET	9	0.713	Hsiao et al. 2008	2µM
2	APT001	178-206	FAM	4	0.034	Hsiao et al. 2008	2µM
2	APT014	317-325	FAM	7	0.662	Hsiao et al. 2008	2µM
2	Apl12	112-155	NED	11	0.410	Denk et al. 2004	2µM
2	CAUD004	199-221	NED	11	0.486	Huang et al. 2005	2µM
2	CAUD014	113-117	PET	5	0.303	Huang et al. 2005	2µM
2	CAUD035	223-237	PET	8	0.614	Huang et al. 2005	2µM

Part of the mitochondrial DNA (mtDNA) gene region, NADH dehydrogenase II (ND2), was sequenced for inferences of sex-biased mating between Mallard and Yellow-billed Ducks. PCR amplification was done using the primers L5216 and H5766 (Table 3b, Sorenson et al., 1999). PCR amplification was performed in a 30 µl reaction mixture containing 15 ng.µl^{-1} of DNA, 1.5 mM $MgCl_2$, 0.5 µM of each primer, 0.2 mM dNTPs (Thermo Fisher Scientific, Waltham, Massachusetts, United States), 0.2 mg.ml^{-1} BSA (Promega, Madison, Wisconsin, United States), 3 µl of 10 × PCR reaction buffer and 0.1 U.µl^{-1} of *Taq* polymerase (Super-Therm JMR-801; Hoffman-La Roche, Basel, Switzerland). PCR conditions followed an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 58

°C for 1 min, elongation at 72 °C for 1 min and a final elongation at 72 °C for 10 min. PCR products were run on a 1% agarose gel to check for amplification. PCR products were purified with the QIAquick PCR purification kit (Qiagen), and submitted for sequencing at the Central Analytical Facility (Stellenbosch University, Stellenbosch, South Africa) using the forward primer only.

Table 3b: Details of primers used for sequencing of mtDNA gene region

Type of DNA	Gene region	Primer name	Sequence	Reference
mtDNA	ND2	H5766	GGATGAGAAGGCTAGGATTTTKCG	Sorenson et al. 1999
		L5216	GCCCATACCCCRAAAATG	

Genetic structure

The full microsatellite data set was checked for null alleles using Micro-Checker version 2.2.3 (Van Oosterhout et al., 2004). Null alleles were detected and consequently corrected (i.e., without null alleles using the ENA method described in Chapuis and Estoup (2007)) and uncorrected estimates of pairwise F_{ST} values (Weir, 1996) were calculated using the software FreeNA (Chapuis & Estoup, 2007). An analysis of variance (ANOVA) was used to test for a significant difference between corrected and uncorrected pairwise F_{ST} values in the R statistical environment version 3.5.1 (R Development Core Team, 2017). There was no significant difference detected ($F_{1,88} = 0.254$, $p = 0.615$) and a low mean null allele frequency ($\bar{x} = 0.050$, standard deviation = 0.091), and therefore all further analyses were conducted using uncorrected data.

Samples that had 50% or more missing data were removed from all further downstream analyses, resulting in a data set consisting of 355 samples (Barberspan (n = 197); Strandfontein (n = 26); Mallard Control Program (n = 110) and museum samples (n = 22)). This data was tested for allele frequency departures from Hardy-Weinberg equilibrium (HWE) expectations using the package pegas (version 0.11, Paradis, 2010) in R (R Development Core Team, 2017). The program STRUCTURE version 2.3.4 (Pritchard et al., 2000) was used to determine the number of genetic clusters (K) and to identify putative hybrid individuals based on the assignment to the inferred number of optimal genetic clusters. Assignment values (q_{ik}) in STRUCTURE are calculated as the proportion of an individual's genotype that belongs to each

genetic cluster. Values of *K* from 1 to 10 were tested using an admixture model with correlated allele frequencies, 100,000 burn-in iterations, 1,000,000 Markov Chain Monte Carlo repetitions, and 20 iterations per run. I used STRUCTURE HARVESTER version 0.6.94 (Earl & vonHoldt, 2012) to determine to optimum number of *K* using the delta K method of Evanno et al. (2005) (Supplementary data Figure S3 and Figure S4). DISTRUCT (version 1.1, Rosenberg, 2004) and a Principal Components Analysis using the R (R Development Core Team, 2017) packages ade4 (version 1.7-11, Thioulouse et al., 1997) and adegraphics (version 1.0-10, Siberchicot et al., 2017) were used to visualise population genetic structure.

Identification of hybrids and assignment to genotype classes

Assignment values (q_{ik}) retrieved in STRUCTURE are dependent on the validity of the assumed priors and the loci used. Consequently, to confirm the identification of hybrid individuals and to assign every individual to a specific genotype class (see Table 3c), I used the program NEWHYBRIDS (version 1.1 beta; Anderson & Thompson, 2002). NEWHYBRIDS is a model-based Bayesian clustering method that uses multilocus genetic data to identity hybrid individuals by computing the posterior probability of each individual belonging to each hybrid genotype class (Anderson & Thompson, 2002). It does not require reference samples of parental individuals (Anderson & Thompson, 2002). I considered six genotype classes, i.e. pure Mallard Duck, pure Yellow-billed Duck, F1, F2, Mallard Duck backcross, and Yellow-billed Duck backcross, in the initial analysis. No prior information regarding the class of individuals was used. I used Jeffery's prior for both theta (allele frequencies) and pi (mixing proportion) with a burn-in of 30,000 MCMC sweeps, followed by 50,000 MCMC sweeps. Individuals were assigned to a genotype class if the posterior probability was greater than or equal to 0.8 (this is the same threshold used in other studies (e.g. see Harrison et al., 2017)); if no genotype class had a greater than 0.8 posterior probability, the individual was considered a hybrid of mixed ancestry. A second analysis was conducted excluding the F2 genotype class and a third analysis including a third generation of hybrid crosses (Table 3d). The genotype classes from the analysis that excluded the F2 genotype class were used for all further analyses because the analysis was able to distinguish backcrossed individuals.

Table 3c: Genotype frequency classes used for the program NEWHYBRIDS. Each column represents the proportion of loci originating from either species

Genotype Frequency classes				
Mallard	1.00	0.00	0.00	0.00
Yellow-billed Duck	0.00	0.00	0.00	1.00
F1	0.00	0.50	0.50	0.00
F2	0.25	0.25	0.25	0.25
Mallard Duck Backcross	0.50	0.25	0.25	0.00
Yellow-billed Duck Backcross	0.00	0.25	0.25	0.50

Table 3d: Genotype frequency classes including a third generation of hybrid classes. Used for the program NEWHYBRIDS. Each column represents the proportion of loci originating from either species

Genotype Frequency classes				
Mallard	1.00	0.00	0.00	0.00
Yellow-billed Duck	0.00	0.00	0.00	1.00
F1	0.00	0.50	0.50	0.00
F2	0.25	0.25	0.25	0.25
Mallard Duck Backcross	0.50	0.25	0.25	0.00
Yellow-billed Duck Backcross	0.00	0.25	0.25	0.50
Mallard Duck Backcross × Mallard Duck	0.75	0.125	0.125	0.00
Yellow-billed Duck Backcross × Yellow-billed Duck	0.00	0.125	0.125	0.75
Mallard Duck Backcross × Yellow-billed Duck	0.25	0.125	0.125	0.50
Yellow-billed Duck Backcross × Mallard Duck	0.50	0.125	0.125	0.25

Simulated genotype dataset

No F1 individuals were identified by NEWHYBRIDS, consequently, to validate the genotype classes assigned by NEWHYBRIDS, I created and analysed a simulated dataset to determine 95% confidence intervals for assignment values (q_{ik}) to a specific species for each genotype class. I selected 39 individuals for each parental population representing pure Mallard Ducks and pure Yellow-billed Ducks that had a greater than or equal to 0.99 assignment value (q_{ik}) to the Mallard or Yellow-billed Duck genetic cluster. A genotype dataset of 100 individuals was simulated for each genotype class (F1 hybrids, F2 hybrids, F1 hybrids backcrossed with

Mallard Ducks and F1 hybrids backcrossed with Yellow-billed Ducks) using the function *hybridize* in the R package *adegenet* (version 2.1.1, Jombart, 2008; R Development Core Team, 2017). Simulated genotypes were analysed in STRUCTURE using the same settings as for the full dataset but limiting *K* to two clusters. 95% confidence intervals were calculated for each simulated genotype.

Assessing directionality of introgression

I estimated the rate of gene flow between Mallard and Yellow-billed Ducks using BayesAss (version 3.0.4, Wilson & Rannala, 2003). I used the mixing parameters dM = 0.05, dA = 0.15, dF = 0.15. The number of MCMC iterations was 1,000,000 with a burn-in of 10,000 and a sampling interval of 1000. I defined the Mallard Duck group as individuals classified by NEWHYBRIDS (analysis excluding the F2 genotype) as Mallard Duck or Mallard Duck Backcross and the Yellow-billed Duck group as individuals classified as Yellow-billed Duck or Yellow-billed Duck Backcross.

DNA sequencing

Pure Mallard and Yellow-billed Duck individuals were sequenced, i.e. individuals with a probability value greater than 0.99 of being assigned to the each class according to the NEWHYBRIDS analysis. Three individuals were sequenced for each species. Thirty two hybrid individuals were sequenced with 17 being assigned to a specific hybrid genotype by NEWHYRIDS (backcrossed Mallard Duck, backcrossed Yellow-billed Duck or F2). Sequences were edited and aligned in BioEdit (version 7.2.5, Hall, 1999). The "pure" individuals were used to determine whether the mitochondrial haplotype was of Mallard Duck or Yellow-billed Duck origin.

Results

Genetic structure

Four loci showed departure from HWE for at least three of the populations that I used for the analysis. This is likely because the populations do not meet the assumptions of no migration and gene flow. Bayesian assignment analysis identified two genetic clusters, corresponding to a Mallard Duck and Yellow-billed Duck cluster. Pure Mallard Ducks (assignment value (q_{ik}) greater than or equal to 0.99) contained 31 private alleles whereas Pure Yellow-billed Ducks had 29 private alleles (Table 3e). The STRUCTURE analysis indicated many instances, and various levels of admixture between these two species - indicative of extensive hybridization and introgression (Figure 3a). Specifically, 26 putative hybrid individuals (assignment value

(q_{ik}) to either genetic cluster <0.9) were identified in the samples obtained from the ongoing Mallard Duck control program in Cape Town. Eight hybrid individuals were also found in the Yellow-billed Duck populations at Strandfontein and Barberspan indicating that hybridization is occurring in these populations. There were also two hybrids identified in the museum samples.

Confirmation of hybrids and assignment to genotype classes

The majority of the samples were in agreement in their genotype assignments across the STRUCTURE and NEWHYBRIDS results (see Supplementary data S4).

Table 3e: Allelic frequencies and private alleles for each species: Pure Mallard and Yellow-billed Duck (assignment value (q_{ik}) greater than or equal to 0.99)

Locus	Allele	Mallard Duck	Yellow-billed Duck
Smo7	183	0.263	0.000
	185	0.113	0.518
	187	0.625	0.012
	189	0.000	0.325
	191	0.000	0.015
	193	0.000	0.130
CAUD026	133	0.000	0.010
	135	0.000	0.159
	138	0.025	0.325
	141	0.075	0.000
	145	0.000	0.052
	147	0.638	0.000
	149	0.238	0.435
	151	0.025	0.000
	153	0.000	0.006
	155	0.000	0.013
CAUD0005	243	0.000	0.003
	247	0.382	0.857
	249	0.132	0.019
	250	0.079	0.000
	253	0.026	0.000
	255	0.026	0.000
	259	0.184	0.000
	261	0.000	0.035
	263	0.013	0.067
	267	0.053	0.013
	272	0.000	0.006
	283	0.092	0.000
	286	0.013	0.000
CAUD031	116	0.513	0.000
	118	0.000	0.110
	120	0.000	0.066
	122	0.000	0.331
	124	0.000	0.051
	126	0.250	0.007
	130	0.013	0.368
	132	0.145	0.066
	140	0.079	0.000
APT004	282	0.000	0.057
	285	0.346	0.000
	289	0.000	0.021
	293	0.013	0.113
	297	0.385	0.504
	301	0.141	0.231
	305	0.115	0.074
APT015	114	0.000	0.031
	118	0.000	0.056
	122	0.054	0.099
	126	0.081	0.182
	130	0.405	0.478
	134	0.338	0.133

	138	0.081	0.019
	142	0.000	0.003
	146	0.041	0.000
APT001	177	0.888	1.000
	185	0.088	0.000
	205	0.025	0.000
APT014	306	0.000	0.003
	310	0.000	0.070
	314	0.463	0.383
	318	0.163	0.215
	322	0.375	0.285
	326	0.000	0.035
	330	0.000	0.009
Apl12	111	0.211	0.000
	115	0.092	0.994
	117	0.368	0.006
	143	0.013	0.000
	153	0.303	0.000
	155	0.013	0.000
CAUD004	196	0.016	0.000
	200	0.313	0.000
	202	0.172	0.886
	204	0.016	0.063
	206	0.203	0.000
	208	0.094	0.000
	210	0.031	0.000
	214	0.016	0.019
	216	0.000	0.016
	224	0.141	0.000
	226	0.000	0.016
CAUD014	116	0.013	0.000
	118	0.526	0.919
	120	0.461	0.064
	122	0.000	0.017
CAUD035	226	0.423	0.000
	228	0.269	0.164
	230	0.000	0.827
	232	0.000	0.009
	234	0.051	0.000
	236	0.141	0.000
	238	0.115	0.000
Private Alleles		31	29

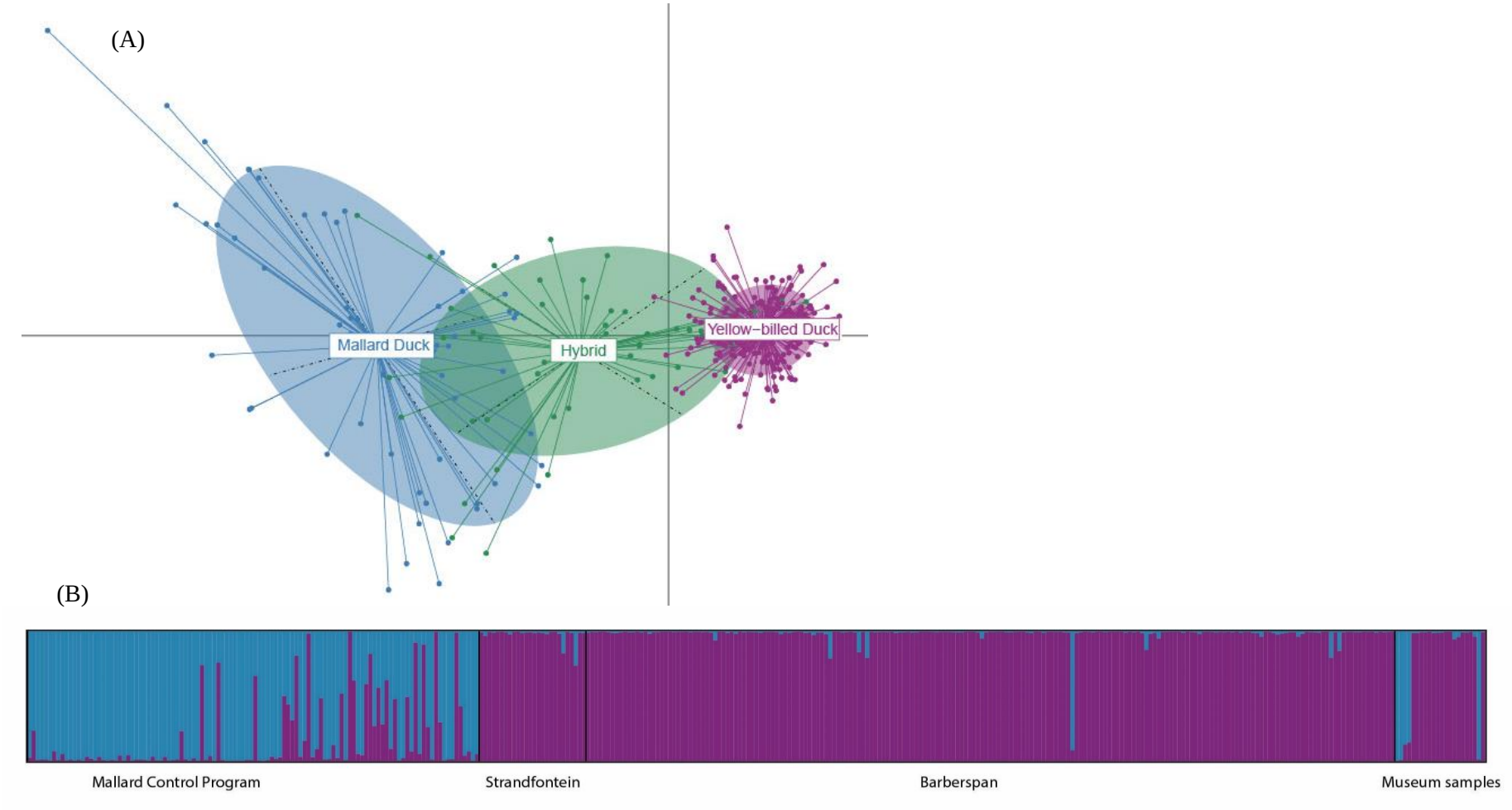

Figure 3a, (A) Scatter plot of Principle Components Analysis showing genetic structure between Mallard Ducks (blue), Yellow-billed Ducks (purple) and putative hybrids (green) (PCA axis 1 = 9.1%; PCA axis 2 = 3.2%). Individuals are grouped by phenotype classification. (B) Structure bar plot where each bar represents an individual and the colour of the bar indicates the proportion of assignment to each genetic cluster (K = 2), Mallard Duck (blue) and Yellow-billed Duck (purple) respectively. The individuals are organised by population.

In total, I identified 68 putative hybrid individuals using NEWHYBRIDS (analysis with the genotype class F2 excluded) (Figure 3b). Of these, none were F1 individuals, 22 had a posterior probability >0.8 of being backcrossed to either Mallard or Yellow-billed Ducks, 19 were classified as Mallard Duck backcrosses, and three as Yellow-billed Duck backcrosses. The remaining 46 individuals could not be assigned to a genotype class with high levels of certainty and were therefore classified as hybrids of mixed ancestry.

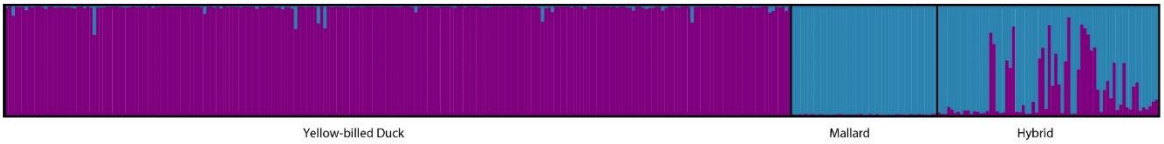

Figure 3b, Structure bar plot of the same data displayed in Figure 3a but arranged in order of genotype assigned by NEWHYBRIDS. Each bar represents an individual and the colour of the bar indicates the proportion of assignment to each genetic cluster (K = 2), Mallard Duck (blue) and Yellow-billed Duck (purple) respectively.

Sixty-three percent of individuals obtained from the control program in Cape Town represented Mallard Duck × Yellow-billed Duck hybrids. Five individuals that were initially classified as hybrid based on morphology (yellow pigment on the feet) are most likely Yellow-billed Ducks according to NEWHYBRIDS (mean posterior probability of being assigned to the Yellow-billed Duck genotype class across all five individuals: $\bar{x}$ =0.983). Natural Yellow-billed Duck populations (Strandfontein and Barberspan) had a high mean posterior probability of being assigned as pure individuals ($\bar{x}$ =0.979). However, three hybrid individuals were identified in these populations, indicating that hybridization is occurring in the Yellow-billed Duck populations. There was also evidence of hybridization in the museum samples; two samples were classified as Mallard Duck backcrosses. Both samples were collected in KwaZulu-Natal in 2009. There was only one sample that seemed to be incorrectly classified by STRUCTURE and NEWHYBRIDS, a museum sample that phenotypically resembled Yellow-billed Duck but was classified as Mallard Duck with a high probability (P = 0.854).

Simulated genotype dataset

My simulation results supported the genotype classes determined by NEWHYBRIDS. When applying the confidence intervals from the simulated data to the actual data, only a few individuals fell into the 95% confidence interval for F1 and F2 assignment values (q_{ik}) for assignment to the Mallard Duck cluster (Figure 3c). Additionally, the simulation indicated that

it is difficult to distinguish between F1 and F2 individuals as the confidence intervals overlapped. The majority of the samples fell outside the confidence intervals supporting that there is extensive backcrossing occurring and that it is likely that most hybrid individuals are representative of several generations of backcrossing.

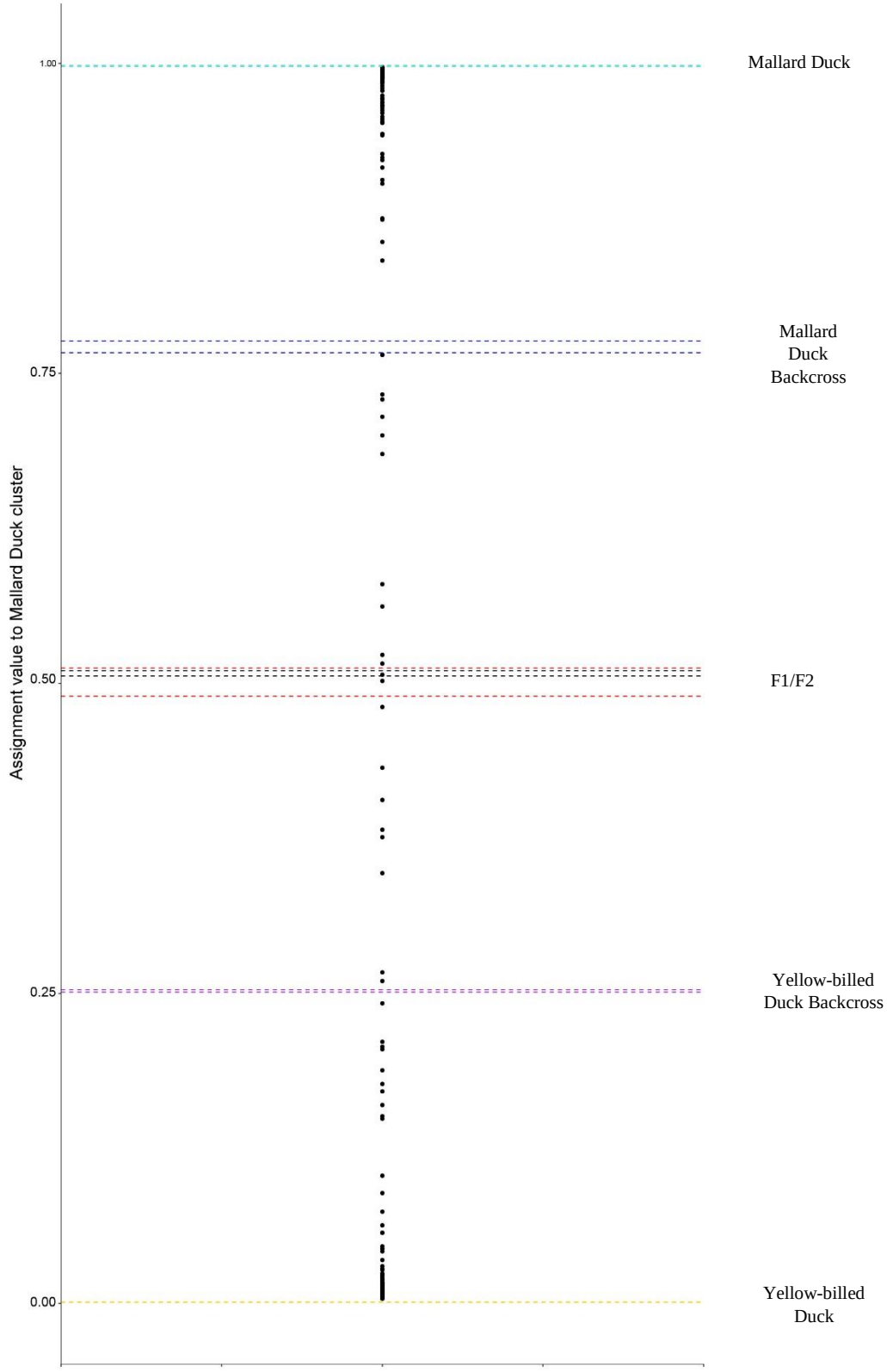

Figure 3c, Actual field-collected data from STRUCTURE plotted against assignment to the Mallard Duck cluster. Dashed lines indicate 95% confidence intervals generated from the simulated data. Confidence interval were generated for six genotypes: Mallard Duck (turquoise), Yellow-billed Duck (yellow), Mallard Duck backcross (blue), Yellow-billed Duck backcross (purple), F2 (red) and F1 (black).

Assessing directionality of introgression

Gene flow from Mallard Ducks into Yellow-billed Ducks was estimated to be 0.0039 migrants per generation (95% credible interval -0.001 to 0.009), while gene flow from Yellow-billed Ducks to Mallard Ducks was much higher (0.0136 migrants per generation, 95% credible interval -0.005 to 0.033). Gene flow from Mallard Ducks into Yellow-billed Ducks was significantly lower than gene flow from Yellow-billed Ducks into Mallard Ducks (Wilcoxon signed-rank test, $P < 0.001$, $Z = 23084$, $n = 1000$ MCMC samples).

DNA sequencing

Mallard and Yellow-billed Ducks were easily distinguished by two consistent, and species-specific, synapomorphies at two positions (sequence divergence 0.0036) within the ND2 mtDNA region. All hybrid individuals that were sequenced carried the Mallard Duck ND2 haplotype (see Supplementary data S4). Similarly, based on ND2 haplotypes, three individuals that were classified as Yellow-billed Duck backcrosses must have resulted from a cross between a Mallard Duck hen × Yellow-billed Duck drake F1, and subsequent backcross with a Yellow-billed Duck drake (Figure 3d). Potential F1 individuals are also the results of a Mallard Duck hen × Yellow-billed Duck drake cross. For the rest of the hybrids that were sequenced, I cannot be certain if the original parents followed the same parental contributions because the hybrids were unable to be classified into a hybrid genotype class. Given that all sequenced samples were carriers of the Mallard Duck ND2 haplotype, it is reasonable to assume that most hybridization events involve Mallard Duck hens.

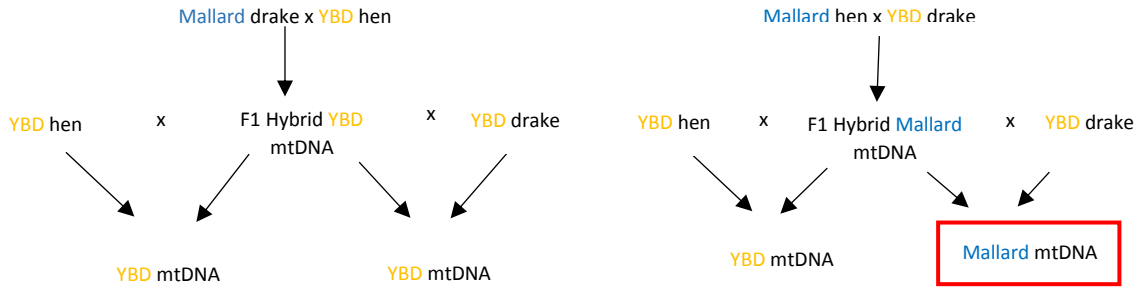

Figure 3d, Diagram illustrating possible hybrid crosses that would result in Yellow-billed Duck backcrosses. Mallard Duck (Mallard) and Yellow-billed Duck (YBD) mitochondrial DNA (mtDNA) haplotypes are indicated. Only one possible combination of crosses can result in a backcrossed Yellow-billed Duck with a Mallard Duck mtDNA haplotype.

Discussion

In support of my first hypothesis, this study supports previous and anecdotal evidence in suggesting that hybridization does occur between invasive Mallard Ducks and native Yellow-billed Ducks in South Africa. Surprisingly, introgression into Yellow-billed Duck populations was not as extensive, with the majority of backcrossed individuals being Mallard Duck backcrosses, and the direction of introgression being rather into the Mallard Duck gene pool, therefore rejecting my second hypothesis. My hypothesis regarding the directionality of sex-biased mating was also not supported, as I clearly illustrated that hybridization is primarily due to mating between Mallard Duck hens and Yellow-billed Duck drakes, rather than the other way around.

Hybridization and introgression

Overall, my findings provide a positive outlook for the long-term genetic integrity for Yellow-billed Ducks in South Africa. Introgression of Mallard Duck genes into Yellow-billed Duck populations appears to be limited, but rather occurs into the Mallard Duck gene pool. My findings are in contrast to most other cases of hybridization between Mallard Ducks and other *Anas* species across the world (Rhymer et al., 1994; Mank et al., 2004; Williams et al., 2005; Fowler et al., 2009). In most of these cases significant hybridization and introgression has occurred between Mallard Ducks and native species. For example, there is a high degree of gene flow between Mallard Ducks and American Black Ducks indicative of significant introgression (Mank et al., 2004), and there is introgression into the native duck population in New Zealand Grey Ducks (Rhymer et al., 1994) and Mottled Ducks (*Anas fulvigula*, Williams et al., 2005). Given the negative outlook due to hybridization between Mallard Ducks and these species, the limited introgression (only three Yellow-billed Duck Backcrossed individuals) found here is surprising. I would caution against optimism, and recommend follow-up studies to ascertain whether the patterns I found here hold up across South Africa or change over time. Mallard Ducks have had longer to establish in the other countries where introgression is more extensive. Mallard Ducks were established in New Zealand in the late 1800s (Dyer & Williams, 2010) and are native to the western United States, having expanded their range eastward in the late 1800s (likely when hybridization began to occur with the American Black Duck) (Johnsgard, 1961; Brodsky & Weatherhead, 1984; Heusmann, 1991). Thus, the level of introgression could be a consequence of residence time as Mallard Ducks have only established fairly recently in South Africa (1940s). However, more research is needed to confirm this hypothesis. Studies using museum samples in these countries where introgression is more

extensive, could help to establish whether the level of introgression has changed over time. According to the precautionary principle, it would be advisable to remove Mallard Ducks and their hybrids while it is likely that control efforts will still have a chance of success. Although there is currently limited introgression in the Yellow-billed Duck population, introgression may become more extensive in South Africa in the future.

Sex-biased mating

Given the reproductive behaviour and ecology of Mallard Ducks, I expected to find sex-biased mating between Mallard Ducks and Yellow-billed Ducks, with most mating occurring between Yellow-billed Duck hens and Mallard Duck drakes. Specifically, Mallard Duck drakes are known to be aggressive when mating (Seymour, 1990; D'Eon et al., 1994), and they are able to outcompete congeneric species for the ability to mate with the hens of those species (e.g. American Black Ducks; Brodsky & Weatherhead, 1984). However, I found the opposite, with most hybridization resulting from mating between Yellow-billed Duck drakes and Mallard Duck hens. Mating between Mallard Duck hens and Yellow-billed Duck drakes has been observed in the Western Cape (Western Cape Birding, 2013). There is also observational evidence of mating between Mallard Duck drakes and Yellow-billed Duck hens, but there is little genetic evidence that these couplings are successful or happening in significant numbers to impact the Yellow-billed Duck genepool. Other studies have also found evidence for sex-biased mating with preferential mating between Mallard Duck hens and drakes of other *Anas* species (Rhymer et al., 1994; Kulikova et al., 2004; Fowler et al., 2009). Kulikova et al. (2004) found that most hybridization resulted from matings between Eastern Spot-billed Duck (*A. zonorhyncha*) drakes and Mallard Duck hens. Similarly, in New Zealand and Hawaii, the majority of Mallard Duck × New Zealand Grey Duck and Hawaiian Duck × Mallard Duck hybrids respectively, carried Mallard Duck mtDNA (Rhymer et al., 1994; Fowler et al., 2009). The reason for this sex-biased mating may be due to a relaxed mate preference by Mallard Duck hens (Fowler et al., 2009). However, I have not tested this hypothesis and so do not know what is the likely cause of the asymmetric contribution towards hybridization in South Africa. The evidence from Russia, New Zealand, Hawaii (Rhymer et al., 1994; Kulikova et al., 2004; Fowler et al., 2009), and now South Africa, does contradict the assumption that Mallard Duck drakes are the primary initiators of hybridization with closely related species. This should be taken into consideration when managing hybridization between Mallard Ducks and other ducks as the hens are more likely to be the initiators of hybridization and should therefore be prioritised during control and eradication attempts. However, before the removal of Mallard

Duck hens is prioritised, potential unintended consequences should be considered. Therefore, due to the uncertainty about the potential consequences of prioritising one sex for removal, the best practice would be removal of all Mallard Ducks.

The sex-biased mating that I found also supports the directionality of introgression inferred here. If most hybridization occurs between Mallard Duck hens and Yellow-billed Duck drakes, the young are likely to be raised within the Mallard Duck population (Steyn, 1996; Doherty et al., 2002). Therefore, hybrid offspring are more likely to mate with Mallard Duck individuals within their own populations (Kruijt, Bossema, & Lammers, 1982). Field observations indicated that Mallard and Yellow-billed Ducks tend to remain in separate populations, making it likely that hybrid offspring would have limited contact with Yellow-billed Ducks.

Implications for control

When individuals are identified for removal during management programs, such as Cape Town's Mallard Duck control program, morphology is usually used to distinguish putative hybrids from pure individuals. However, I found five individuals that were identified as hybrid using morphology (having yellow pigment on their feet) that were genetically identified as Yellow-billed Ducks. Identifying hybrid individuals using morphological characteristics alone can be very difficult (Allendorf et al., 2001; Jombart et al., 2008). In this case, although these Yellow-billed Ducks were potentially euthanised unnecessarily. The remaining population is large and widespread (The IUCN Red List, 2016; South African Bird Atlas Project 2, 2018b), so removing a few individuals will not have a major impact on the population. Furthermore, it is better to remove individuals that could be "pure" Yellow-billed Ducks than risk not removing potential hybrids. This is a real risk considering that backcrossed individuals can often be indistinguishable from parental species (Rhymer & Simberloff, 1996; Devillard et al., 2014). I also had individuals with a hybrid genotype that were misidentified as Mallard Ducks based on morphology alone, but such misidentifications should not be a concern for a control program, as both hybrids and Mallard Ducks are targeted. The accuracy of identifying hybrid ducks using morphological characteristics could be a topic for further research as it could help with identifying individuals to be removed through control programs, particularly backcrossed individuals that may be located in the Yellow-billed Duck populations and that are difficult to identify.

Is hybridization still a threat to the Yellow-billed Duck?

Although levels of hybridization between Mallard and Yellow-billed Ducks are not as severe as expected, there was evidence of backcrossing. There was a low number of F1 and F2 individuals indicating that a lot of backcrossing is occurring rather than hybridization between parental individuals. In addition, most hybrid individuals could not be assigned to a genotype class with a high level of certainty and they were classified as hybrids of mixed ancestry. This uncertainty most likely reflects that the markers were not informative enough and therefore the number of markers used was not enough to distinguish hybrids resulting from multiple generations of backcrossing. When using NEWHYBRIDS as many as 48 loci can be necessary to correctly assign 95% of backcrossed individuals (Vähä & Primmer, 2006). To be able to detect backcrossing the use of at least 18 loci would be advisable (with an F_{ST} of at least 0.21) as the percentage of correctly assigned backcrossed individuals increases to around 80% when the number of loci is increased from 12 to 18 (Vähä & Primmer, 2006). However, most hybrids resulting from several generations of backcrossing are likely to remain unclassified as they are very difficult to distinguish from parental individuals and other hybrid genotype classes (Allendorf et al., 2001; Vähä & Primmer, 2006) due to the large number of potential crosses from which they could have resulted.

The evidence of several generations of backcrossing and the identification of three Yellow-billed Backcrossed individuals, indicates that there is still a potential threat to the Yellow-billed Duck population as introgression could become more extensive in the future. Thus, the removal of Mallard Ducks and hybrid individuals is still necessary to protect the genetic integrity of Yellow-billed Ducks. I did find some hybrid individuals, albeit a few, in the Yellow-billed Duck populations. Also, some museum samples were identified as hybrids, indicating that hybridization has been occurring since at least 2009 in KwaZulu-Natal. This, in combination with the hybrid identified at Barberspan, indicates that hybridization between the two species is occurring in other areas of South Africa outside of Cape Town. Research on the extent of hybridization in other areas of South Africa where Mallard Ducks occur (especially in areas where they are concentrated like Gauteng) is therefore needed. Additionally, although most Yellow-billed Ducks have local movements of 50 km or less (Brown et al., 1983), some Yellow-billed Duck individuals have been recorded to fly distances of over 1000 km (Hockey, Dean, & Ryan, 2005). Mallard Ducks are also known to migrate long distances in their native range (Hoyo, Elliott, & Sargatal, 1992). Therefore, it is likely that hybridization could spread through these movements and consequently, control programs will also be needed in the other

areas of South Africa where hybridization has been recorded. Without a national effort, the control program in Cape Town will not be enough to protect the genetic integrity of the Yellow-billed Duck.

Conclusion

Evidence of hybridization and backcrossing between invasive Mallard Ducks and native Yellow-billed Ducks in South Africa substantiates the need to remove Mallard Ducks. Although there is currently a low level of introgression of Mallard Duck genes into the Yellow-billed Duck gene pool, introgression could become more extensive in the future. It is thus advisable to remove Mallard Ducks while such interventions will have a high chance of success. That is, unlike other native *Anas* species impacted by Mallard Duck hybridization around the world, e.g. New Zealand Grey Duck, there is still a good chance of protecting the long-term genetic integrity South Africa's Yellow-billed Duck through the removal of Mallard Ducks and their hybrids. My hope is that when this message is communicated effectively to the communities where Mallard Duck removal efforts continue, that it will sway the public's perception in favour of the control of this invasive duck.

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Chapter Four

Thesis conclusions

Mallard Ducks threaten the native Yellow-billed Duck in South Africa through hybridization and introgression. Hybridization and introgression can reduce genetic uniqueness, and in some instances even lead to the extinction of native genotypes and co-adapted gene complexes. However, there has been public opposition to the control of Mallard Ducks in South Africa, partly due to a lack of awareness of the potential threat that Mallard Ducks pose to the native Yellow-billed Duck. Despite the threat from hybridization, the perceived hybridization between the two species was based on observational and anecdotal evidence, with no solid scientific evidence to support hybridization between the two species.

In Chapter Two, I obtained baseline genetic data for the Yellow-billed Duck in South Africa, in light of the threat posed by hybridization with the Mallard Duck. This data was used to determine if the current Yellow-billed Duck populations represent a panmictic population and to use historical data to help with understanding the impacts of different pressures on the Yellow-billed Duck population over the last 60 years.

I showed that the current Yellow-billed Duck populations represent a panmictic population with enough migration between populations to prevent the formation of a strong population genetic structure. This has two important aspects to consider for the conservation of the Yellow-billed Duck. Firstly, it indicates that there is no evidence of distinct genetic diversity in local populations, so there is no need to focus resources on conserving any specific population of Yellow-billed Ducks. Secondly, in terms of the threat from hybridization with the Mallard Duck, the evidence of migration between populations indicates that there is potential for hybrid genes to spread between Yellow-billed Duck populations.

Historical genetic data showed that the genetic diversity and structure of the Yellow-billed Duck has not had any major changes over the last 60 years, suggesting that the Yellow-billed Duck has not had any significant demographic changes. This means that it is likely that recent threats such as urbanisation and habitat destruction have not negatively impacted the Yellow-billed Duck population in South Africa as a whole.

In Chapter Three I used microsatellite genotyping and sequencing of a mitochondrial gene region to investigate if hybridization and introgression were occurring between Mallard and Yellow-billed Ducks and if sex-biased mating was occurring. There is evidence of

hybridization and introgression between the two species, but most backcrossing and introgression is occurring into the Mallard Duck population. Therefore, the Yellow-billed Duck population is currently largely unaffected by introgression but introgression could still become more extensive in the future. In terms of sex-biased mating, most mating is occurring between Mallard Duck hens and Yellow-billed Duck drakes. This means that Mallard Duck hens could be prioritised for removal and that it would be best to remove Mallard Ducks while there is still a high chance of success of protecting the genetic integrity of the Yellow-billed Duck. Unlike other native species impacted by hybridization with the Mallard Duck, introgression is limited, which means there is still a good chance of protecting the long-term genetic integrity of South Africa's Yellow-billed Duck through the removal of Mallard Ducks and their hybrids. My hope is that these findings will help to convince the public in favour of the control of this invasive duck.

My study therefore has both positive and negative implications for the threat of hybridization between Mallard and Yellow-billed Ducks. Limited introgression into the Yellow-billed Duck population is positive news because it means that Mallard Duck control efforts will have a good chance of success in protecting the long-term genetic integrity of South Africa's Yellow-billed Duck population. Nonetheless, this situation may change as introgression could become more prominant in the future. Additionally, research has yet to be done on the extent of hybridization in other regions of southern Africa where Mallard Ducks have been recorded, and so, I advise that further research be conducted in these areas. Of concern is the finding that there is potential for hybridization to spread through migration over long distances. Therefore, managers should continue to monitor Yellow-billed Duck populations for the potential spread of hybrid genes and control efforts should be implemented at a national level in areas where hybridization has already been recorded.

The key findings of this study show that genetic techniques are valuable for the management of hybridization between invasive species and native congeners. This study was valuable in providing insights on the hybridization between Mallard and Yellow-billed Ducks, which would not have been determined with observational evidence alone. The main finding of this study is that it would be advisable to continue control efforts in South Africa in order to remove Mallard Ducks while there is still a good chance of protecting the genetic integrity of the Yellow-billed Duck.

Supplementary material

Chapter Two

Table S1: All samples included in this study, including details on location, year of collection and the population. A = analysis; BAR = Barberspan; STR = Strandfontein; MDG = Marina Da Gama; BMM = Bloemfontein Museum; DMM = Durban Museum; ELM = East London Museum; IZM = Iziko Museum; MMK = McGregor Museum

Analysis code	Sample name	Location	Year collected	Population
A146	BAR1450	Barberspan, North West Province	2013	Barberspan
A147	BAR1657	Barberspan, North West Province	2013	Barberspan
A148	BAR1443	Barberspan, North West Province	2013	Barberspan
A149	BAR1900	Barberspan, North West Province	2014	Barberspan
A150	BAR2044	Barberspan, North West Province	2014	Barberspan
A151	BAR1550	Barberspan, North West Province	2013	Barberspan
A152	BAR1448	Barberspan, North West Province	2013	Barberspan
A153	BAR2068	Barberspan, North West Province	2014	Barberspan
A155	BAR1594	Barberspan, North West Province	2013	Barberspan
A156	BAR1706	Barberspan, North West Province	2013	Barberspan
A157	BAR1954	Barberspan, North West Province	2014	Barberspan
A159	BAR2073	Barberspan, North West Province	2014	Barberspan
A160	BAR1606	Barberspan, North West Province	2013	Barberspan
A164	BAR1707	Barberspan, North West Province	2013	Barberspan
A165	BAR1914	Barberspan, North West Province	2014	Barberspan
A166	BAR1879	Barberspan, North West Province	2014	Barberspan
A167	BAR2046	Barberspan, North West Province	2014	Barberspan
A168	BAR1620	Barberspan, North West Province	2013	Barberspan
A171	BAR1407	Barberspan, North West Province	2013	Barberspan

A172	BAR1628	Barberspan, North West Province	2013	Barberspan
A173	BAR1881	Barberspan, North West Province	2014	Barberspan
A174	BAR2042	Barberspan, North West Province	2014	Barberspan
A175	BAR1897	Barberspan, North West Province	2014	Barberspan
A176	BAR2061	Barberspan, North West Province	2014	Barberspan
A177	BAR1705	Barberspan, North West Province	2013	Barberspan
A178	BAR1421	Barberspan, North West Province	2013	Barberspan
A179	BAR1949	Barberspan, North West Province	2014	Barberspan
A180	BAR1660	Barberspan, North West Province	2013	Barberspan
A181	BAR2038	Barberspan, North West Province	2014	Barberspan
A182	BAR1480	Barberspan, North West Province	2013	Barberspan
A183	BAR1608	Barberspan, North West Province	2013	Barberspan
A184	BAR1697	Barberspan, North West Province	2013	Barberspan
A185	BAR1663	Barberspan, North West Province	2013	Barberspan
A186	BAR1712	Barberspan, North West Province	2013	Barberspan
A187	BAR1475	Barberspan, North West Province	2013	Barberspan
A188	BAR1683	Barberspan, North West Province	2013	Barberspan
A189	BAR2043	Barberspan, North West Province	2014	Barberspan
A190	BAR2000	Barberspan, North West Province	2014	Barberspan
A191	BAR1452	Barberspan, North West Province	2013	Barberspan
A193	BAR1713	Barberspan, North West Province	2013	Barberspan
A195	BAR1873	Barberspan, North West Province	2014	Barberspan
A196	BAR1953	Barberspan, North West Province	2014	Barberspan
A197	BAR1611	Barberspan, North West Province	2013	Barberspan
A198	BAR1951	Barberspan, North West Province	2014	Barberspan
A199	BAR1446	Barberspan, North West Province	2013	Barberspan
A200	BAR1485	Barberspan, North West Province	2013	Barberspan
A201	BAR1610	Barberspan, North West Province	2013	Barberspan

A202	BAR1679	Barberspan, North West Province	2013	Barberspan
A203	BAR1502	Barberspan, North West Province	2013	Barberspan
A204	BAR1872	Barberspan, North West Province	2014	Barberspan
A207	BAR1876	Barberspan, North West Province	2014	Barberspan
A212	BAR1537	Barberspan, North West Province	2013	Barberspan
A217	BAR1871	Barberspan, North West Province	2014	Barberspan
A218	BAR1895	Barberspan, North West Province	2014	Barberspan
A219	BAR2080	Barberspan, North West Province	2014	Barberspan
A221	BAR1875	Barberspan, North West Province	2014	Barberspan
A224	BAR1969	Barberspan, North West Province	2014	Barberspan
A226	BAR1414	Barberspan, North West Province	2013	Barberspan
A227	BAR1908	Barberspan, North West Province	2014	Barberspan
A233	BAR2036	Barberspan, North West Province	2014	Barberspan
A235	BAR1512	Barberspan, North West Province	2013	Barberspan
A236	BAR1490	Barberspan, North West Province	2013	Barberspan
A239	BAR1878	Barberspan, North West Province	2014	Barberspan
A245	BAR1868	Barberspan, North West Province	2014	Barberspan
A247	BAR1435	Barberspan, North West Province	2013	Barberspan
A250	BAR1909	Barberspan, North West Province	2014	Barberspan
A258	BAR1978	Barberspan, North West Province	2014	Barberspan
A259	BAR2032	Barberspan, North West Province	2014	Barberspan
A260	BAR1866	Barberspan, North West Province	2014	Barberspan
A261	BAR1472	Barberspan, North West Province	2013	Barberspan
A263	BAR2089	Barberspan, North West Province	2014	Barberspan
A264	BAR1826	Barberspan, North West Province	2014	Barberspan
A266	BAR1509	Barberspan, North West Province	2013	Barberspan
A268	BAR1874	Barberspan, North West Province	2014	Barberspan
A274	BAR1997	Barberspan, North West Province	2014	Barberspan

A275	BAR1677	Barberspan, North West Province	2013	Barberspan
A276	BAR1925	Barberspan, North West Province	2014	Barberspan
A281	BAR1535	Barberspan, North West Province	2013	Barberspan
A284	BAR1545	Barberspan, North West Province	2013	Barberspan
A285	BAR2019	Barberspan, North West Province	2014	Barberspan
A290	BAR2002	Barberspan, North West Province	2014	Barberspan
A291	BAR1966	Barberspan, North West Province	2014	Barberspan
A295	BAR2065	Barberspan, North West Province	2014	Barberspan
A296	BAR2059	Barberspan, North West Province	2014	Barberspan
A297	BAR1968	Barberspan, North West Province	2014	Barberspan
A298	BAR2070	Barberspan, North West Province	2014	Barberspan
A299	BAR1528	Barberspan, North West Province	2013	Barberspan
A300	BAR1500	Barberspan, North West Province	2013	Barberspan
A301	BAR1515	Barberspan, North West Province	2013	Barberspan
A303	BAR2057	Barberspan, North West Province	2014	Barberspan
A304	BAR2071	Barberspan, North West Province	2014	Barberspan
A305	BAR2072	Barberspan, North West Province	2014	Barberspan
A306	BAR1585	Barberspan, North West Province	2013	Barberspan
A307	BAR1681	Barberspan, North West Province	2013	Barberspan
A308	BAR1455	Barberspan, North West Province	2013	Barberspan
A312	BAR1870	Barberspan, North West Province	2014	Barberspan
A313	BAR1698	Barberspan, North West Province	2013	Barberspan
A314	BAR1882	Barberspan, North West Province	2014	Barberspan
A315	BAR2003	Barberspan, North West Province	2014	Barberspan
A316	BAR1569	Barberspan, North West Province	2013	Barberspan
A317	BAR2045	Barberspan, North West Province	2014	Barberspan
A318	BAR1454	Barberspan, North West Province	2013	Barberspan
A319	BAR1533	Barberspan, North West Province	2013	Barberspan

A320	BAR1630	Barberspan, North West Province	2013	Barberspan
A321	BAR2035	Barberspan, North West Province	2014	Barberspan
A322	BAR1516	Barberspan, North West Province	2013	Barberspan
A323	BAR1482	Barberspan, North West Province	2013	Barberspan
A324	BAR1643	Barberspan, North West Province	2013	Barberspan
A325	BAR2069	Barberspan, North West Province	2014	Barberspan
A326	BAR1711	Barberspan, North West Province	2013	Barberspan
A327	BAR2031	Barberspan, North West Province	2014	Barberspan
A329	BAR1588	Barberspan, North West Province	2013	Barberspan
A330	BAR2004	Barberspan, North West Province	2014	Barberspan
A331	BAR1568	Barberspan, North West Province	2013	Barberspan
A332	BAR1419	Barberspan, North West Province	2013	Barberspan
A333	BAR1913	Barberspan, North West Province	2014	Barberspan
A334	BAR2067	Barberspan, North West Province	2014	Barberspan
A337	BAR1665	Barberspan, North West Province	2013	Barberspan
A339	BAR1655	Barberspan, North West Province	2013	Barberspan
A340	BAR1488	Barberspan, North West Province	2013	Barberspan
A342	BAR1479	Barberspan, North West Province	2013	Barberspan
A343	BAR1513	Barberspan, North West Province	2013	Barberspan
A346	BAR1662	Barberspan, North West Province	2013	Barberspan
A348	BAR2054	Barberspan, North West Province	2014	Barberspan
A349	BAR2037	Barberspan, North West Province	2014	Barberspan
A350	BAR1656	Barberspan, North West Province	2013	Barberspan
A351	BAR1530	Barberspan, North West Province	2013	Barberspan
A354	BAR1603	Barberspan, North West Province	2013	Barberspan
A355	BAR2005	Barberspan, North West Province	2014	Barberspan
A356	BAR1896	Barberspan, North West Province	2014	Barberspan
A358	BAR1800	Barberspan, North West Province	2014	Barberspan

A362	BAR1514	Barberspan, North West Province	2013	Barberspan
A363	BAR1551	Barberspan, North West Province	2013	Barberspan
A364	BAR1441	Barberspan, North West Province	2013	Barberspan
A365	BAR2074	Barberspan, North West Province	2014	Barberspan
A366	BAR1459	Barberspan, North West Province	2013	Barberspan
A367	BAR1946	Barberspan, North West Province	2014	Barberspan
A368	BAR2058	Barberspan, North West Province	2014	Barberspan
A373	BAR2018	Barberspan, North West Province	2014	Barberspan
A375	BAR1631	Barberspan, North West Province	2013	Barberspan
A376	BAR1511	Barberspan, North West Province	2013	Barberspan
A377	BAR1642	Barberspan, North West Province	2013	Barberspan
A378	BAR2034	Barberspan, North West Province	2014	Barberspan
A379	BAR1604	Barberspan, North West Province	2013	Barberspan
A380	BAR1415	Barberspan, North West Province	2013	Barberspan
A381	BAR1531	Barberspan, North West Province	2013	Barberspan
A382	BAR1449	Barberspan, North West Province	2013	Barberspan
A383	BAR2047	Barberspan, North West Province	2014	Barberspan
A384	BAR2048	Barberspan, North West Province	2014	Barberspan
A385	BAR2090	Barberspan, North West Province	2014	Barberspan
A386	BAR1478	Barberspan, North West Province	2013	Barberspan
A387	BAR1473	Barberspan, North West Province	2013	Barberspan
A388	BAR1661	Barberspan, North West Province	2013	Barberspan
A389	BAR1477	Barberspan, North West Province	2013	Barberspan
A390	BAR1474	Barberspan, North West Province	2013	Barberspan
A391	BAR1653	Barberspan, North West Province	2013	Barberspan
A392	BAR1710	Barberspan, North West Province	2013	Barberspan
A394	BAR1709	Barberspan, North West Province	2013	Barberspan
A397	BAR1442	Barberspan, North West Province	2013	Barberspan

A399	BAR2041	Barberspan, North West Province	2014	Barberspan
A401	BAR1899	Barberspan, North West Province	2014	Barberspan
A402	BAR2079	Barberspan, North West Province	2014	Barberspan
A403	BAR1527	Barberspan, North West Province	2013	Barberspan
A404	BAR1420	Barberspan, North West Province	2013	Barberspan
A410	BAR1680	Barberspan, North West Province	2013	Barberspan
A411	BAR2053	Barberspan, North West Province	2014	Barberspan
A412	BAR1916	Barberspan, North West Province	2014	Barberspan
A414	BAR1567	Barberspan, North West Province	2013	Barberspan
A415	BAR1447	Barberspan, North West Province	2013	Barberspan
A416	BAR1549	Barberspan, North West Province	2013	Barberspan
A417	BAR1977	Barberspan, North West Province	2014	Barberspan
A418	BAR1919	Barberspan, North West Province	2014	Barberspan
A420	BAR1481	Barberspan, North West Province	2013	Barberspan
A421	BAR2033	Barberspan, North West Province	2014	Barberspan
A422	BAR1491	Barberspan, North West Province	2013	Barberspan
A423	BAR1867	Barberspan, North West Province	2014	Barberspan
A424	BAR2017	Barberspan, North West Province	2014	Barberspan
A425	BAR1704	Barberspan, North West Province	2013	Barberspan
A426	BAR1413	Barberspan, North West Province	2013	Barberspan
A427	BAR1907	Barberspan, North West Province	2014	Barberspan
A429	BAR1676	Barberspan, North West Province	2013	Barberspan
A430	BAR1492	Barberspan, North West Province	2013	Barberspan
A432	BAR1621	Barberspan, North West Province	2013	Barberspan
A433	BAR1584	Barberspan, North West Province	2013	Barberspan
A434	BAR1566	Barberspan, North West Province	2013	Barberspan
A436	BAR1880	Barberspan, North West Province	2014	Barberspan
A437	BAR1508	Barberspan, North West Province	2013	Barberspan

A438	BAR1487	Barberspan, North West Province	2013	Barberspan
A440	BAR2016	Barberspan, North West Province	2014	Barberspan
A441	BAR1658	Barberspan, North West Province	2013	Barberspan
A443	BAR1476	Barberspan, North West Province	2013	Barberspan
A445	BAR1629	Barberspan, North West Province	2013	Barberspan
A446	BAR1678	Barberspan, North West Province	2013	Barberspan
A450	BAR1453	Barberspan, North West Province	2013	Barberspan
A451	BAR1501	Barberspan, North West Province	2013	Barberspan
A158	STR0984	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A170	STR0956	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A194	STR0942	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A208	STR1024	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A213	STR0973	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A220	STR0994	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A229	STR1100	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A238	STR1092	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A240	STR1070	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A243	STR1061	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A246	STR1071	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A251	STR1085	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A256	STR1110	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A267	STR1034	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A270	STR0981	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A335	STR0971	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A341	STR1101	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A345	STR1068	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A357	STR1053	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A393	STR0964	Strandfontein Sewer Works, Cape Town	2014	Strandfontein

A405	STR1023	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A406	STR0995	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A413	STR0968	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A435	STR0962	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A439	STR1069	Strandfontein Sewer Works, Cape Town	2014	Strandfontein
A085	MDG00A51	Marina Da Gama, Cape Town	2018	Marina Da Gama
A100	MDG0057	Marina Da Gama, Cape Town	2018	Marina Da Gama
A123	MDG0015	Marina Da Gama, Cape Town	2018	Marina Da Gama
A131	MDG0043	Marina Da Gama, Cape Town	2018	Marina Da Gama
A138	MDG0040	Marina Da Gama, Cape Town	2018	Marina Da Gama
A280	BMM2540	Bloemfontein (Bloemfontein National Museum)	1987	Museum samples
A255	DMM0020	Old Durban Airport (Durban Natural Science Museum)	1999	Museum samples
A257	DMM0014	Old Durban Airport (Durban Natural Science Museum)	1999	Museum samples
A241	ELM4537	Happy Valley, Eastern Cape (East London Museum)	1957	Museum samples
A278	DMM0007	Mamathes, Lesotho (Durban Natural Science Museum)	1956	Museum samples
A210	DMM0008	Ladysmith, KwaZulu-Natal (Durban Natural Science Museum)	1977	Museum samples
A234	DMM0003	Ladysmith, KwaZulu-Natal (Durban Natural Science Museum)	1970	Museum samples
A249	DMM0015	Mitchell Park Zoo, KwaZulu-Natal (Durban Natural Science Museum)	1964	Museum samples

A214	DMM0011	Mooi River, Sarsgrove Farm, KwaZulu-Natal (Durban Natural Science Museum)	1962	Museum samples
A242	DMM0002	Mooi River, Sarsgrove Farm, KwaZulu-Natal (Durban Natural Science Museum)	1962	Museum samples
A248	DMM0009	Mooi River, Sarsgrove Farm, KwaZulu-Natal (Durban Natural Science Museum)	1962	Museum samples
A253	DMM0006	Mooi River, Sarsgrove Farm, KwaZulu-Natal (Durban Natural Science Museum)	1962	Museum samples
A277	DMM0024	Mooi River, Sarsgrove Farm, KwaZulu-Natal (Durban Natural Science Museum)	1962	Museum samples
A231	DMM0013	Richards Bay, Thulazihleka Pan, KwaZulu-Natal (Durban Natural Science Museum)	1999	Museum samples
A455	IZM2536	Rondevlei, Western Cape (Iziko Museum)	1957	Museum samples
A215	MMK1569	Rooipoort, North West Province (McGregor Museum)	1982	Museum samples
A286	DMM0001	Victoria Falls, Kazangula Ranch, Zimbabwe (Durban Natural Science Museum)	1967	Museum samples

Table S2: Original set of microsatellite primers selected for PCR optimization

Primer name	Forward primer	Reverse Primer	Repeat unit	Reference
APT016	TCT TAA ATG GGA CTG ATG GAG AGA G	ACC TAT TTT ATC TCA GGA TGC AAT TAT G	(GATA)10	Hsiao et al. 2008
APT013	CCA ACC ACC AGG AAG TAC TGT AAA TA	AGG AAA GTT CAG ACA CAT GGA TTG	(GATA)10	Hsiao et al. 2008
APT018	GTG GCA GTT TAATGA AAG CGA AA	TGG AGG TAC CCA AAG GAG AAT TC	(GATA)9(GAAA)14(GA)2 (GAAA)2 (GA)6(GAAA)2	Hsiao et al. 2008
APT014	GCA CCA GGT AAT TTA TGT CAG AAA TAA T	GAA GTG CAA AAC ATG GTT CAG G	(GATA)11	Hsiao et al. 2008
APT006	CTT CCC ATT GCA GTG TTG GTC	TTG GCA TCT TTG TTC TGC AGA	(GATA)12	Hsiao et al. 2008
CAUD030	ATTATTCCTGATGGCGTGGT	TCATGCTGAATTTGGCTGTT	(CA)9. . . (AT)6T10	Huang et al. 2005
CAUD018	TTAGACAAATGAGGAAATAGTA	GTCCAAACTAAATGCAGGC	(CA)9(CA)9	Huang et al. 2005
CAUD031	AGCATCTGGACTTTTTCTGGA	CACCCCAGGCTCTGAGATAA	(TTTC)9(TC)25	Huang et al. 2005
APT015	CTG TTA TGA CAC CAT GTT TGG ATT TA	CGT GCT CTG CAA CAA CTG AAA	(GATA)13	Hsiao et al. 2008

APT028	CAT TCA TGT TTA TTT CTT CTG GTA TGT G	GTT AAA ATG GGA AGG CTT CAC TAG A	(GATA)10	Hsiao et al. 2008
APT001	GTC CCA CTG GTT TGC TGT CC	ACT ACG CAT GGC AGT GAG GTT	(GATA)12	Hsiao et al. 2008
APT032	TCA CTT TCT TGA CTC TCC TTG GTT T	TGA CTT GAA TTC TGT TCA GGA TAA ATG	GATA(GACA)2GACT (GATA)4	Hsiao et al. 2008
APT003	GAT CAT TGC ACT TGA AAT TAT TGT TAT TT	TGT GCA TTA CTG TGG CAG ATC TG	(GATA)11	Hsiao et al. 2008
APT005	TCC GTA CAG ACC AAC ATC GG	AGG TCT TTA CAG CCC ACT CCC	(GATA)17	Hsiao et al. 2008
APT004	GGG CAG GAA AAT CTC CTG AAT	TCT CAG TGG CTG AGC GGT C	GATAGAT(GATA)15	Hsiao et al. 2008
APT009	CCA GGC AGT TGC TGT GTA ACA	GGC GCT TTC TTC TAT GAT CGA	(GATA)2GAT(GATA)15	Hsiao et al. 2008
CAUD024	TCGCATTAAGCTCTGATCT	ATCAACAGAATCCAAAATATG	(TTTC)33. . . T19	Huang et al. 2005
CAUD014	CACAACTGACGGCACAAAGT	CTGAGTTTTTCCCGCCTCTA	(AC)7GC(AC)6	Huang et al. 2005
CAUD032	GAAACCAACTGAAAACGGGC	CCTCCTGCGTCCCAATAAG	(CA)n	Huang et al. 2005
CAUD033	ACCCAGAAGAGTCAAGAATAG	GAGTATTCCTGGTCTGTGCT	(AC)10. . . T9	Huang et al. 2005
CAUD013	ACAATAGATTCCAGATGCTGAA	ATGTCTGAGTCCTCGGAGC	(AC)25	Huang et al. 2005
Apl12	AGTTGACCCTAATGTCAGCATC	AAGAGACACTGAGAAGTGCTATTG	$(GA)_{27}$	Denk et al. 2004
Smo7	TTTTCACCCAGTTCACTTCAGCC	GATTCAAATTTGCCGCAGGATTA	(GT)12	Paulus & Tiedemann, 2003
Apl11	AACTACAGGGCACCTTATTTCC	TTGCATCAGGGTCTGTATTTTC	$(GA)_{25}$	Denk et al. 2004
CAUD026	ACGTCACATCACCCCACAG	CTTTGCCTCTGGTGAGGTTC	(AC)17	Huang et al. 2005
CAUD004	TCCACTTGGTAGACCTTGAG	TGGGATTCAGTGAGAAGCCT	(AC)20	Huang et al. 2005

CAUD005	CTGGGTTTGGTGGAGCATAA	TACTGGCTGCTTCATTGCTG	(TC)18	Huang et al. 2005
CAUD035	GTGCCTAACCCTGATGGATG	CTTATCAGATGGGGCTCGGA	(CA)n	Huang et al. 2005

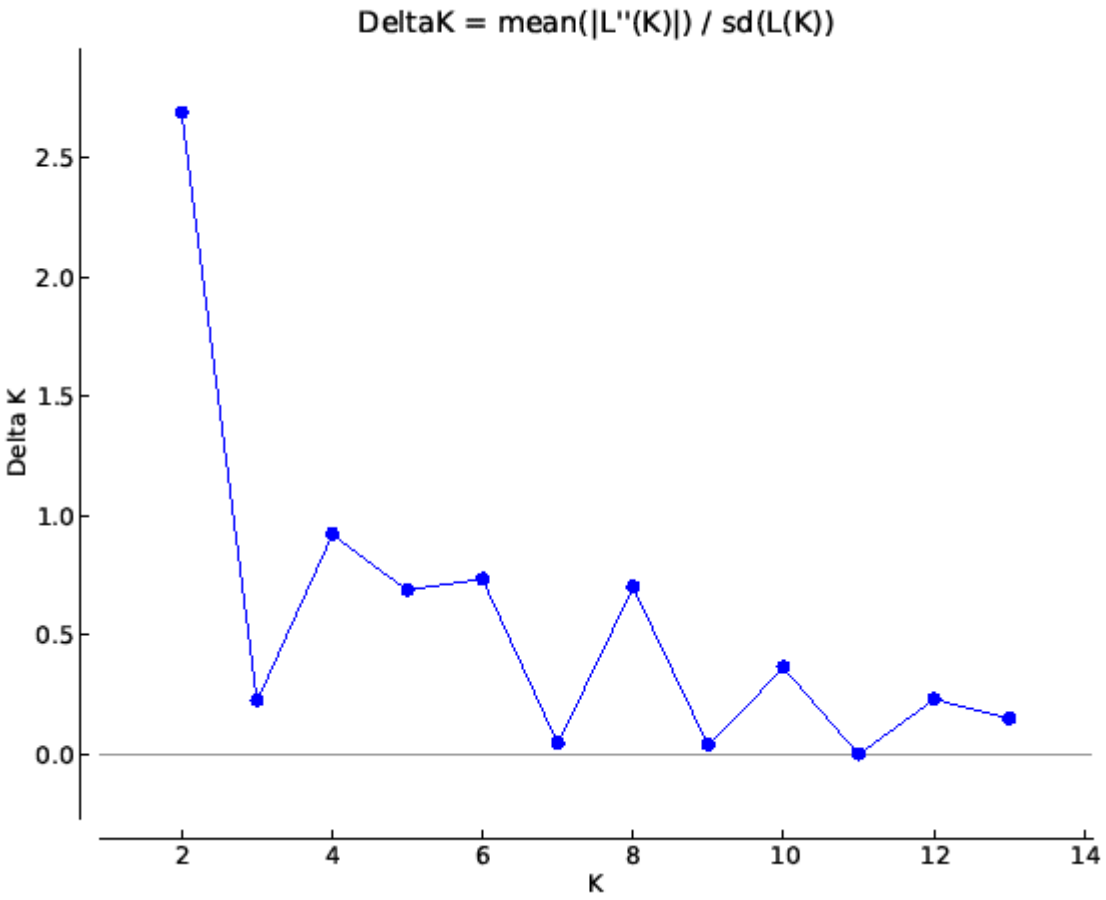

Figure S1, Delta K plot to determine the optimal number of genetic clusters

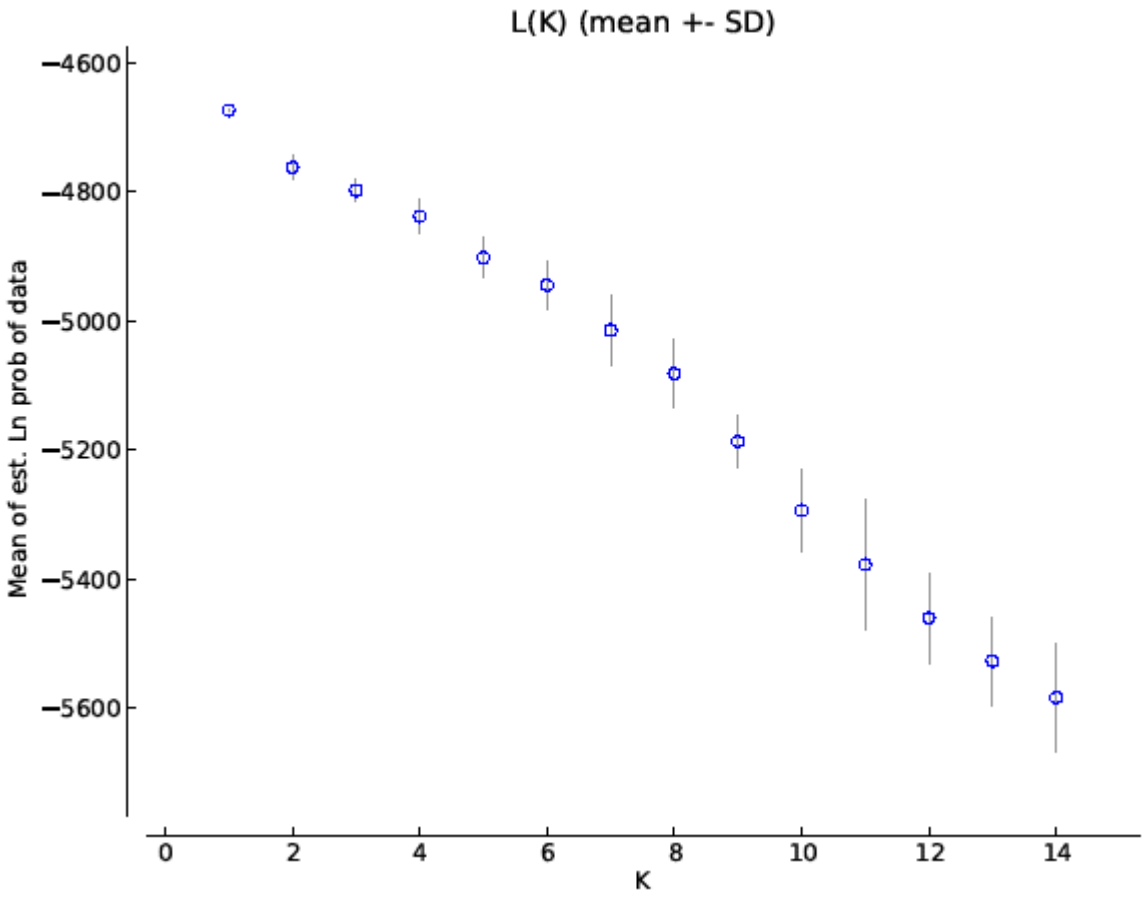

Figure S2, Mean Log probability plot to determine the optimal number of genetic clusters

Chapter Three

Table S3: All samples collected for this study, including details on location, collection method, year of collection and whether the sample was used in the analysis; AGG = Arderne Gardens; DPP = Dam Park; MGC = Milnerton Golf Course; MDG = Marina Da Gama; WPP = Wynberg Park; BAR = Barberspan; STR = Strandfontein; BMM = Bloemfontein Museum; DMM = Durban Museum; ELM = East London Museum; IZM = Iziko Museum; MMK = McGregor Museum

Sample Name	Location	Collection Method	Year Collected	Used in Final Analysis
AGG0001	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0002	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0003	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0004	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0005	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0007	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0008	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0009	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0010	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0012	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0013	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0014	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0015	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0016	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0017	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
AGG0018	Arderne Gardens, Cape Town	Mallard Control Program	2017	Yes
DPP0035	Dam Park, Cape Town	Mallard Control Program	2016	Yes
DPP0006	Dam Park, Cape Town	Mallard Control Program	2015	No
DPP0012	Dam Park, Cape Town	Mallard Control Program	2015	Yes
DPP0030	Dam Park, Cape Town	Mallard Control Program	2016	Yes
DPP0031	Dam Park, Cape Town	Mallard Control Program	2016	Yes
DPP0032	Dam Park, Cape Town	Mallard Control Program	2016	Yes

DPP0034	Dam Park, Cape Town	Mallard Control Program	2016	Yes
DPP0036	Dam Park, Cape Town	Mallard Control Program	2016	Yes
DPP0037	Dam Park, Cape Town	Mallard Control Program	2016	Yes
DPP0038	Dam Park, Cape Town	Mallard Control Program	2016	Yes
DPP0039	Dam Park, Cape Town	Mallard Control Program	2016	Yes
MGC0003	Milnerton Golf Course, Cape Town	Mallard Control Program	2017	Yes
MDG0001	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0002	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0003	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0004	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0005	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0006	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0007	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0008	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0009	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0010	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0011	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0012	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0013	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0014	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0015	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0016	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0018	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0019	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0020	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0021	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0022	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0023	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0024	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0025	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes

MDG0026	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0027	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0028	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0029	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0030	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0031	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0032	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0033	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0034	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0035	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0036	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0037	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0038	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0039	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0040	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0041	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0042	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0043	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0044	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0045	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0046	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0047	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0048	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0049	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0050	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0051	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0052	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0053	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0054	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0055	Marina Da Gama, Cape Town	Mallard Control Program	2018	No

MDG0056	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0057	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0058	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0059	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0060	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0061	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0062	Marina Da Gama, Cape Town	Mallard Control Program	2018	No
MDG0063	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0064	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0065	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0066	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0067	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0068	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0069	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0070	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0071	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0072	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0073	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0074	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0076	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0077	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0078	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0080	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0081	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0083	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0084	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0085	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0086	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0087	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0088	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes

MDG0089	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0090	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0091	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
MDG0092	Marina Da Gama, Cape Town	Mallard Control Program	2018	Yes
WPP0001	Wynberg Park, Cape Town	Mallard Control Program	2017	Yes
WPP0005	Wynberg Park, Cape Town	Mallard Control Program	2017	Yes
WPP0006	Wynberg Park, Cape Town	Mallard Control Program	2017	Yes
WPP0010	Wynberg Park, Cape Town	Mallard Control Program	2017	Yes
WPP0017	Wynberg Park, Cape Town	Mallard Control Program	2017	Yes
BAR1407	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1413	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1414	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1415	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	No
BAR1419	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1420	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1421	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1435	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1441	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1442	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1443	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1446	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1447	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1448	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1449	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1450	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1452	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1453	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1454	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1455	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1459	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes

BAR1472	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1473	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1474	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1475	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1476	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1477	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1478	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1479	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1480	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1481	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1482	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1484	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	No
BAR1485	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1487	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1488	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1489	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	No
BAR1490	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1491	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1492	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1500	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1501	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1502	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1508	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1509	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1511	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1512	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1513	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1514	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1515	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1516	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes

BAR1527	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1528	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1530	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1531	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1533	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1535	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1537	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1545	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1549	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1550	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1551	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1566	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1567	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1568	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1569	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1584	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1585	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1588	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1594	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1603	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1604	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1606	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1608	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1610	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1611	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1620	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1621	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1628	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1629	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1630	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes

BAR1631	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1642	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1643	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1653	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1655	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1656	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1657	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1658	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1660	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1661	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1662	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1663	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1665	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1676	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1677	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1678	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1679	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1680	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1681	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1683	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1698	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1697	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1704	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1705	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1706	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1707	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1709	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1710	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1711	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1712	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes

BAR1713	Barberspan, North West Province	Donated by Chevonne Reynolds	2013	Yes
BAR1800	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1826	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1865	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1866	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1867	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1868	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1869	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1870	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1871	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1872	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1873	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1874	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1875	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1876	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1878	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1879	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1880	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1881	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1882	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1895	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1896	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1897	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1899	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1900	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1907	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1908	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1909	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1913	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1914	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes

BAR1916	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1919	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1925	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1946	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1949	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1951	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1953	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1954	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1966	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1968	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1969	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1977	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1978	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR1997	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2000	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2002	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2003	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2004	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2005	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2006	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	No
BAR2016	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2017	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2018	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2019	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2031	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2032	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2033	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2034	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2035	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2036	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes

BAR2037	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2038	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2041	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2042	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2043	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2044	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2045	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2046	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2047	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2048	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2053	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2054	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2057	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2058	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2059	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2061	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2065	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2067	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2068	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2069	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2070	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2071	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2072	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2073	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2074	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2079	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2080	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2089	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
BAR2090	Barberspan, North West Province	Donated by Chevonne Reynolds	2014	Yes
STR0942	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes

STR0956	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0959	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0962	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0963	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	No
STR0964	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0966	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	No
STR0968	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0971	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0973	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0981	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0984	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0994	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR0995	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1023	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1024	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1034	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1038	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	No
STR1053	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1061	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1068	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1069	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1070	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1071	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1085	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1091	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	No
STR1092	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1099	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	No
STR1100	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1101	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes
STR1110	Strandfontein Sewer Works, Cape Town	Donated by Chevonne Reynolds	2014	Yes

BMM0021	Glen, Free State	Bloemfontein National Museum	1975	No
BMM0206	Modderpoort, Free State	Bloemfontein National Museum	1977	No
BMM0387	Modderpoort, Free State	Bloemfontein National Museum	1976	No
BMM2540	Bloemfontein	Bloemfontein National Museum	1987	Yes
DMM0012	Mooi River, Sarsgrove Farm, KwaZulu-Natal	Durban Natural Science Museum	1962	No
DMM0001	Victoria Falls, Kazangula Ranch, Zimbabwe	Durban Natural Science Museum	1967	Yes
DMM0002	Mooi River, Sarsgrove Farm, KwaZulu-Natal	Durban Natural Science Museum	1962	Yes
DMM0003	Ladysmith, KwaZulu-Natal	Durban Natural Science Museum	1970	Yes
DMM0004	Matatiele, Eastern Cape	Durban Natural Science Museum	1956	No
DMM0005	Matatiele, Eastern Cape	Durban Natural Science Museum	1956	No
DMM0006	Mooi River, Sarsgrove Farm, KwaZulu-Natal	Durban Natural Science Museum	1962	Yes
DMM0007	Mamathes, Lesotho	Durban Natural Science Museum	1956	Yes
DMM0008	Ladysmith, KwaZulu-Natal	Durban Natural Science Museum	1977	Yes
DMM0009	Mooi River, Sarsgrove Farm, KwaZulu-Natal	Durban Natural Science Museum	1962	Yes
DMM0010	Matatiele, Eastern Cape	Durban Natural Science Museum	1965	No
DMM0011	Mooi River, Sarsgrove Farm, KwaZulu-Natal	Durban Natural Science Museum	1962	Yes
DMM0013	Richards Bay, Thulazihleka Pan, KwaZulu-Natal	Durban Natural Science Museum	1999	Yes
DMM0014	Old Durban Airport	Durban Natural Science Museum	1999	Yes
DMM0015	Mitchell Park Zoo, KwaZulu-Natal	Durban Natural Science Museum	1964	Yes
DMM0016	Kenville Sewerage Works, KwaZulu-Natal	Durban Natural Science Museum	1978	No
DMM0017	Pietermaritzburg	Durban Natural Science Museum	1964	No
DMM0018	Pietermaritzburg	Durban Natural Science Museum	1964	No
DMM0019	Kenville Sewerage Works, KwaZulu-Natal	Durban Natural Science Museum	1978	No
DMM0020	Old Durban Airport	Durban Natural Science Museum	1999	Yes
DMM0021	Mooi River, Sarsgrove Farm, KwaZulu-Natal	Durban Natural Science Museum	1962	No
DMM0022	Bela Vista, Maputo, Mozambique	Durban Natural Science Museum	1960	No
DMM0024	Mooi River, Sarsgrove Farm, KwaZulu-Natal	Durban Natural Science Museum	1962	Yes
DMM0025	Boston area, KwaZulu-Natal	Durban Natural Science Museum	2009	Yes
DMM0026	Cedara College, Pietermaritzburg	Durban Natural Science Museum	2006	Yes
DMM0027	Amanzimtoti Bird Sanctuary, KwaZulu-Natal	Durban Natural Science Museum	2005	No

DMM0028	Blue Crane farm, Mt West, Nottingham Road, KwaZulu-Natal	Durban Natural Science Museum	2009	No
DMM0029	Amanzimtoti Bird Sanctuary, KwaZulu-Natal	Durban Natural Science Museum	2004	No
DMM0030	Mitchell Park Zoo, KwaZulu-Natal	Durban Natural Science Museum	2009	Yes
DMM0031	Blue Crane farm, Mt West, Nottingham Road, KwaZulu-Natal	Durban Natural Science Museum	2009	Yes
DMM0032	Richmond, KwaZulu-Natal	Durban Natural Science Museum	2009	No
ELM1417	Amalinda Dam East London	East London Museum	1969	No
ELM1502	Queen's Park Zoo, East London	East London Museum	1973	No
ELM1512	Horseshoe Valley, Cambridge, East London	East London Museum	1974	No
ELM3402	Mtana River, Peddie district, Eastern Cape	East London Museum	1956	No
ELM4535	Happy Valley, Eastern Cape	East London Museum	1957	No
ELM4536	Happy Valley, Eastern Cape	East London Museum	1957	No
ELM4537	Happy Valley, Eastern Cape	East London Museum	1957	Yes
ELM4538	Happy Valley, Eastern Cape	East London Museum	1957	No
ELM8986	Willowvale, Eastern Cape	East London Museum	1961	No
IZM1058	Matatiele, Eastern Cape	Iziko Museum	1909	Yes
IZM2135	Verlorenvlei, Western Cape	Iziko Museum	1958	No
IZM2532	Rondevlei, Western Cape	Iziko Museum	1959	No
IZM2536	Rondevlei, Western Cape	Iziko Museum	1957	Yes
IZM3703	Malawi	Iziko Museum	n.d.	No
MMK0936	Douglas, Northern Cape	McGregor Museum	1966	No
MMK1569	Rooipoort, North West Province	McGregor Museum	1982	Yes

Table S4: Key results of the analyses for this study: Structure assignment value (q_{ik}) to the Mallard Duck cluster (column 3), Genotype class assigned by NEWHYBRIDS (column 4) and mtDNA haplotype determined from sequencing of the ND2 gene region (column 5)

Sample name	Phenotype	Assignment to Mallard Duck cluster	Genotype class	mtDNA haplotype
AGG0001	Hybrid	0.971	Hybrid	
AGG0002	Hybrid	0.992	Mallard	
AGG0003	Hybrid	0.99	Mallard	
AGG0004	Mallard	0.988	Hybrid	
AGG0005	Hybrid	0.982	Hybrid	
AGG0007	Hybrid	0.99	Mallard	
AGG0008	Mallard	0.989	Hybrid	Mallard
AGG0009	Hybrid	0.96	Hybrid	
AGG0010	Hybrid	0.99	Mallard	
AGG0012	Mallard	0.922	Hybrid	
AGG0013	Mallard	0.988	Mallard	
AGG0014	Hybrid	0.242	Hybrid	
AGG0015	Hybrid	0.347	Hybrid	
AGG0016	Mallard	0.994	Mallard	
AGG0017	Hybrid	0.962	Hybrid	Mallard
AGG0018	Mallard	0.972	Hybrid	Mallard
DPP0012	Mallard	0.993	Mallard	

DPP0030	Mallard	0.996	Mallard	
DPP0031	Mallard	0.996	Mallard	
DPP0032	Mallard	0.996	Mallard	Mallard
DPP0034	Mallard	0.996	Mallard	
DPP0035	Mallard	0.995	Mallard	
DPP0036	Mallard	0.997	Mallard	
DPP0037	Mallard	0.997	Mallard	Mallard
DPP0038	Mallard	0.991	Mallard	
DPP0039	Mallard	0.997	Mallard	Mallard
MGC0003	Hybrid	0.26	BxYBD	Mallard
MDG0001	Mallard	0.964	Hybrid	
MDG0002	Hybrid	0.995	Mallard	
MDG0003	Hybrid	0.733	BxMallard	Mallard
MDG0004	Mallard	0.989	Hybrid	
MDG0005	Mallard	0.955	Hybrid	
MDG0006	Hybrid	0.715	Hybrid	Mallard
MDG0007	Hybrid	0.992	Mallard	
MDG0008	Mallard	0.994	Mallard	
MDG0009	Mallard	0.994	Mallard	
MDG0010	Hybrid	0.516	BxMallard	Mallard

MDG0011	Hybrid	0.985	Hybrid	
MDG0012	Hybrid	0.729	Hybrid	Mallard
MDG0015	Hybrid	0.089	Yellow-billed Duck	
MDG0018	Mallard	0.988	Mallard	
MDG0019	Hybrid	0.903	BxMallard	
MDG0021	Mallard	0.995	Mallard	
MDG0023	Mallard	0.995	Mallard	
MDG0024	Mallard	0.993	Mallard	
MDG0025	Mallard	0.978	Hybrid	
MDG0026	Mallard	0.995	Mallard	
MDG0027	Mallard	0.968	BxMallard	
MDG0028	Mallard	0.98	Hybrid	
MDG0029	Mallard	0.994	Mallard	
MDG0030	Mallard	0.989	Mallard	
MDG0031	Mallard	0.992	Mallard	
MDG0032	Mallard	0.996	Mallard	
MDG0033	Hybrid	0.382	Hybrid	
MDG0034	Mallard	0.994	Mallard	
MDG0035	Mallard	0.99	Mallard	
MDG0036	Hybrid	0.523	BxMallard	

MDG0037	Hybrid	0.985	Hybrid	
MDG0038	Hybrid	0.188	Hybrid	
MDG0039	Hybrid	0.906	Hybrid	
MDG0040	Hybrid	0.012	Yellow-billed Duck	
MDG0041	Hybrid	0.177	Hybrid	
MDG0042	Hybrid	0.988	Mallard	
MDG0043	Hybrid	0.006	Yellow-billed Duck	
MDG0044	Mallard	0.953	Hybrid	
MDG0045	Mallard	0.966	Hybrid	
MDG0047	Hybrid	0.103	Hybrid	Mallard
MDG0048	Hybrid	0.432	Hybrid	Mallard
MDG0050	Hybrid	0.987	Hybrid	
MDG0052	Hybrid	0.974	Hybrid	
MDG0053	Hybrid	0.406	BxYBD	Mallard
MDG0054	Hybrid	0.978	Hybrid	
MDG0056	Mallard	0.993	Mallard	
MDG0057	Hybrid	0.004	Yellow-billed Duck	
MDG0058	Hybrid	0.986	Hybrid	
MDG0059	Mallard	0.99	Hybrid	
MDG0061	Hybrid	0.685	BxMallard	Mallard

MDG0063	Hybrid	0.993	Mallard	
MDG0064	Hybrid	0.993	Mallard	
MDG0065	Hybrid	0.942	BxMallard	Mallard
MDG0066	Hybrid	0.7	BxMallard	Mallard
MDG0067	Mallard	0.994	Mallard	
MDG0068	Mallard	0.991	Mallard	
MDG0069	Hybrid	0.58	Hybrid	Mallard
MDG0070	Hybrid	0.924	BxMallard	Mallard
MDG0071	Mallard	0.966	Hybrid	
MDG0072	Hybrid	0.957	BxMallard	
MDG0073	Hybrid	0.875	Hybrid	
MDG0074	Hybrid	0.966	Hybrid	
MDG0076	Hybrid	0.841	BxMallard	Mallard
MDG0077	Mallard	0.765	BxMallard	
MDG0078	Mallard	0.994	Mallard	
MDG0080	Hybrid	0.927	BxMallard	Mallard
MDG0081	Hybrid	0.562	Hybrid	Mallard
MDG0083	Hybrid	0.952	BxMallard	Mallard
MDG0084	Hybrid	0.376	BxYBD	Mallard
MDG0085	Hybrid	0.502	Hybrid	Mallard

MDG0086	Hybrid	0.943	BxMallard	Mallard
MDG0087	Hybrid	0.969	Hybrid	
MDG0088	Hybrid	0.507	Hybrid	Mallard
MDG0089	Mallard	0.988	Mallard	
MDG0090	Hybrid	0.481	Hybrid	Mallard
MDG0091	Hybrid	0.943	Hybrid	
MDG0092	Mallard	0.984	Hybrid	
MDG00A51	Hybrid	0.017	Yellow-billed Duck	
WPP0001	Mallard	0.991	Mallard	
WPP0005	Mallard	0.943	Hybrid	Mallard
WPP0006	Mallard	0.993	Mallard	
WPP0010	Mallard	0.992	Mallard	
WPP0017	Mallard	0.765	BxMallard	Mallard
BAR1407	Yellow-billed Duck	0.004	Yellow-billed Duck	
BAR1413	Yellow-billed Duck	0.005	Yellow-billed Duck	
BAR1414	Yellow-billed Duck	0.01	Yellow-billed Duck	
BAR1415	Yellow-billed Duck	0.013	Yellow-billed Duck	
BAR1419	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1420	Yellow-billed Duck	0.044	Yellow-billed Duck	
BAR1421	Yellow-billed Duck	0.008	Yellow-billed Duck	

BAR1435	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1441	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1442	Yellow-billed Duck	0.013	Yellow-billed Duck
BAR1443	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1446	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1447	Yellow-billed Duck	0.017	Yellow-billed Duck
BAR1448	Yellow-billed Duck	0.014	Yellow-billed Duck
BAR1449	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1450	Yellow-billed Duck	0.012	Yellow-billed Duck
BAR1452	Yellow-billed Duck	0.01	Yellow-billed Duck
BAR1453	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1454	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1455	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1459	Yellow-billed Duck	0.149	Yellow-billed Duck
BAR1472	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1473	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1474	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1475	Yellow-billed Duck	0.014	Yellow-billed Duck
BAR1476	Yellow-billed Duck	0.012	Yellow-billed Duck
BAR1477	Yellow-billed Duck	0.007	Yellow-billed Duck

BAR1478	Yellow-billed Duck	0.005	Yellow-billed Duck	
BAR1479	Yellow-billed Duck	0.005	Yellow-billed Duck	
BAR1480	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1481	Yellow-billed Duck	0.042	Yellow-billed Duck	
BAR1482	Yellow-billed Duck	0.005	Yellow-billed Duck	
BAR1485	Yellow-billed Duck	0.006	Yellow-billed Duck	Yellow-billed Duck
BAR1487	Yellow-billed Duck	0.005	Yellow-billed Duck	
BAR1488	Yellow-billed Duck	0.009	Yellow-billed Duck	
BAR1490	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1491	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1492	Yellow-billed Duck	0.007	Yellow-billed Duck	
BAR1500	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1501	Yellow-billed Duck	0.004	Yellow-billed Duck	
BAR1502	Yellow-billed Duck	0.007	Yellow-billed Duck	
BAR1508	Yellow-billed Duck	0.007	Yellow-billed Duck	
BAR1509	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1511	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1512	Yellow-billed Duck	0.005	Yellow-billed Duck	
BAR1513	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1514	Yellow-billed Duck	0.005	Yellow-billed Duck	

BAR1515	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1516	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1527	Yellow-billed Duck	0.016	Yellow-billed Duck
BAR1528	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1530	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1531	Yellow-billed Duck	0.004	Yellow-billed Duck
BAR1533	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR1535	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR1537	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1545	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1549	Yellow-billed Duck	0.017	Yellow-billed Duck
BAR1550	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR1551	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR1566	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1567	Yellow-billed Duck	0.028	Yellow-billed Duck
BAR1568	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1569	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1584	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1585	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1588	Yellow-billed Duck	0.014	Yellow-billed Duck

BAR1594	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR1603	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1604	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1606	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1608	Yellow-billed Duck	0.011	Yellow-billed Duck
BAR1610	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1611	Yellow-billed Duck	0.014	Yellow-billed Duck
BAR1620	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1621	Yellow-billed Duck	0.151	Yellow-billed Duck
BAR1628	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1629	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1630	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR1631	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1642	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1643	Yellow-billed Duck	0.01	Yellow-billed Duck
BAR1653	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1655	Yellow-billed Duck	0.015	Yellow-billed Duck
BAR1656	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1657	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1658	Yellow-billed Duck	0.005	Yellow-billed Duck

BAR1660	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1661	Yellow-billed Duck	0.01	Yellow-billed Duck	
BAR1662	Yellow-billed Duck	0.008	Yellow-billed Duck	
BAR1663	Yellow-billed Duck	0.005	Yellow-billed Duck	Yellow-billed Duck
BAR1665	Yellow-billed Duck	0.01	Yellow-billed Duck	
BAR1676	Yellow-billed Duck	0.205	Hybrid	
BAR1677	Yellow-billed Duck	0.014	Yellow-billed Duck	
BAR1678	Yellow-billed Duck	0.008	Yellow-billed Duck	
BAR1679	Yellow-billed Duck	0.024	Yellow-billed Duck	
BAR1680	Yellow-billed Duck	0.004	Yellow-billed Duck	
BAR1681	Yellow-billed Duck	0.004	Yellow-billed Duck	
BAR1683	Yellow-billed Duck	0.006	Yellow-billed Duck	
BAR1697	Yellow-billed Duck	0.074	Yellow-billed Duck	
BAR1698	Yellow-billed Duck	0.057	Yellow-billed Duck	
BAR1704	Yellow-billed Duck	0.013	Yellow-billed Duck	
BAR1705	Yellow-billed Duck	0.005	Yellow-billed Duck	Yellow-billed Duck
BAR1706	Yellow-billed Duck	0.005	Yellow-billed Duck	
BAR1707	Yellow-billed Duck	0.011	Yellow-billed Duck	
BAR1709	Yellow-billed Duck	0.005	Yellow-billed Duck	
BAR1710	Yellow-billed Duck	0.008	Yellow-billed Duck	

BAR1711	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1712	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1713	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1800	Yellow-billed Duck	0.012	Yellow-billed Duck
BAR1826	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1865	Yellow-billed Duck	0.916	BxMallard
BAR1866	Yellow-billed Duck	0.207	Yellow-billed Duck
BAR1867	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1868	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1869	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR1870	Yellow-billed Duck	0.011	Yellow-billed Duck
BAR1871	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1872	Yellow-billed Duck	0.014	Yellow-billed Duck
BAR1873	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR1874	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1875	Yellow-billed Duck	0.03	Yellow-billed Duck
BAR1876	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1878	Yellow-billed Duck	0.011	Yellow-billed Duck
BAR1879	Yellow-billed Duck	0.019	Yellow-billed Duck
BAR1880	Yellow-billed Duck	0.007	Yellow-billed Duck

BAR1881	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1882	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR1895	Yellow-billed Duck	0.014	Yellow-billed Duck
BAR1896	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1897	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1899	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1900	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1907	Yellow-billed Duck	0.004	Yellow-billed Duck
BAR1908	Yellow-billed Duck	0.023	Yellow-billed Duck
BAR1909	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR1913	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1914	Yellow-billed Duck	0.004	Yellow-billed Duck
BAR1916	Yellow-billed Duck	0.017	Yellow-billed Duck
BAR1919	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1925	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR1946	Yellow-billed Duck	0.019	Yellow-billed Duck
BAR1949	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1951	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1953	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1954	Yellow-billed Duck	0.009	Yellow-billed Duck

BAR1966	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR1968	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR1969	Yellow-billed Duck	0.01	Yellow-billed Duck
BAR1977	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR1978	Yellow-billed Duck	0.16	Yellow-billed Duck
BAR1997	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2000	Yellow-billed Duck	0.004	Yellow-billed Duck
BAR2002	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR2003	Yellow-billed Duck	0.004	Yellow-billed Duck
BAR2004	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR2005	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2016	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR2017	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR2018	Yellow-billed Duck	0.057	Yellow-billed Duck
BAR2019	Yellow-billed Duck	0.01	Yellow-billed Duck
BAR2031	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2032	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR2033	Yellow-billed Duck	0.019	Yellow-billed Duck
BAR2034	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2035	Yellow-billed Duck	0.006	Yellow-billed Duck

BAR2036	Yellow-billed Duck	0.211	Yellow-billed Duck
BAR2037	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2038	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR2041	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR2042	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR2043	Yellow-billed Duck	0.021	Yellow-billed Duck
BAR2044	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2045	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2046	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR2047	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR2048	Yellow-billed Duck	0.024	Yellow-billed Duck
BAR2053	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR2054	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2057	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR2058	Yellow-billed Duck	0.014	Yellow-billed Duck
BAR2059	Yellow-billed Duck	0.005	Yellow-billed Duck
BAR2061	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR2065	Yellow-billed Duck	0.006	Yellow-billed Duck
BAR2067	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR2068	Yellow-billed Duck	0.015	Yellow-billed Duck

BAR2069	Yellow-billed Duck	0.017	Yellow-billed Duck
BAR2070	Yellow-billed Duck	0.007	Yellow-billed Duck
BAR2071	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR2072	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR2073	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR2074	Yellow-billed Duck	0.016	Yellow-billed Duck
BAR2079	Yellow-billed Duck	0.015	Yellow-billed Duck
BAR2080	Yellow-billed Duck	0.009	Yellow-billed Duck
BAR2089	Yellow-billed Duck	0.008	Yellow-billed Duck
BAR2090	Yellow-billed Duck	0.006	Yellow-billed Duck
STR0942	Yellow-billed Duck	0.006	Yellow-billed Duck
STR0956	Yellow-billed Duck	0.035	Yellow-billed Duck
STR0959	Yellow-billed Duck	0.171	Hybrid
STR0962	Yellow-billed Duck	0.013	Yellow-billed Duck
STR0964	Yellow-billed Duck	0.02	Yellow-billed Duck
STR0968	Yellow-billed Duck	0.267	Yellow-billed Duck
STR0971	Yellow-billed Duck	0.016	Yellow-billed Duck
STR0973	Yellow-billed Duck	0.006	Yellow-billed Duck
STR0981	Yellow-billed Duck	0.01	Yellow-billed Duck
STR0984	Yellow-billed Duck	0.008	Yellow-billed Duck

STR0994	Yellow-billed Duck	0.004	Yellow-billed Duck
STR0995	Yellow-billed Duck	0.016	Yellow-billed Duck
STR1023	Yellow-billed Duck	0.006	Yellow-billed Duck
STR1024	Yellow-billed Duck	0.014	Yellow-billed Duck
STR1034	Yellow-billed Duck	0.014	Yellow-billed Duck
STR1053	Yellow-billed Duck	0.005	Yellow-billed Duck
STR1061	Yellow-billed Duck	0.005	Yellow-billed Duck
STR1068	Yellow-billed Duck	0.006	Yellow-billed Duck
STR1069	Yellow-billed Duck	0.014	Yellow-billed Duck
STR1070	Yellow-billed Duck	0.006	Yellow-billed Duck
STR1071	Yellow-billed Duck	0.017	Yellow-billed Duck
STR1085	Yellow-billed Duck	0.01	Yellow-billed Duck
STR1092	Yellow-billed Duck	0.027	Yellow-billed Duck
STR1100	Yellow-billed Duck	0.009	Yellow-billed Duck
STR1101	Yellow-billed Duck	0.022	Yellow-billed Duck
STR1110	Yellow-billed Duck	0.008	Yellow-billed Duck
BMM2540	Yellow-billed Duck	0.006	Yellow-billed Duck
DMM0001	Yellow-billed Duck	0.044	Yellow-billed Duck
DMM0002	Yellow-billed Duck	0.012	Yellow-billed Duck
DMM0003	Yellow-billed Duck	0.005	Yellow-billed Duck

DMM0006	Yellow-billed Duck	0.006	Yellow-billed Duck
DMM0007	Yellow-billed Duck	0.012	Yellow-billed Duck
DMM0008	Yellow-billed Duck	0.014	Yellow-billed Duck
DMM0009	Yellow-billed Duck	0.01	Yellow-billed Duck
DMM0011	Yellow-billed Duck	0.015	Yellow-billed Duck
DMM0013	Yellow-billed Duck	0.009	Yellow-billed Duck
DMM0014	Yellow-billed Duck	0.046	Yellow-billed Duck
DMM0015	Yellow-billed Duck	0.006	Yellow-billed Duck
DMM0020	Yellow-billed Duck	0.063	Yellow-billed Duck
DMM0024	Yellow-billed Duck	0.008	Yellow-billed Duck
DMM0025	Hybrid	0.856	BxMallard
DMM0026	Mallard	0.99	Mallard
DMM0030	Mallard	0.996	Mallard
DMM0031	Mallard	0.874	BxMallard
ELM4537	Yellow-billed Duck	0.015	Yellow-billed Duck
IZM1058	Yellow-billed Duck	0.988	Mallard
IZM2536	Yellow-billed Duck	0.007	Yellow-billed Duck
MMK1569	Yellow-billed Duck	0.008	Yellow-billed Duck

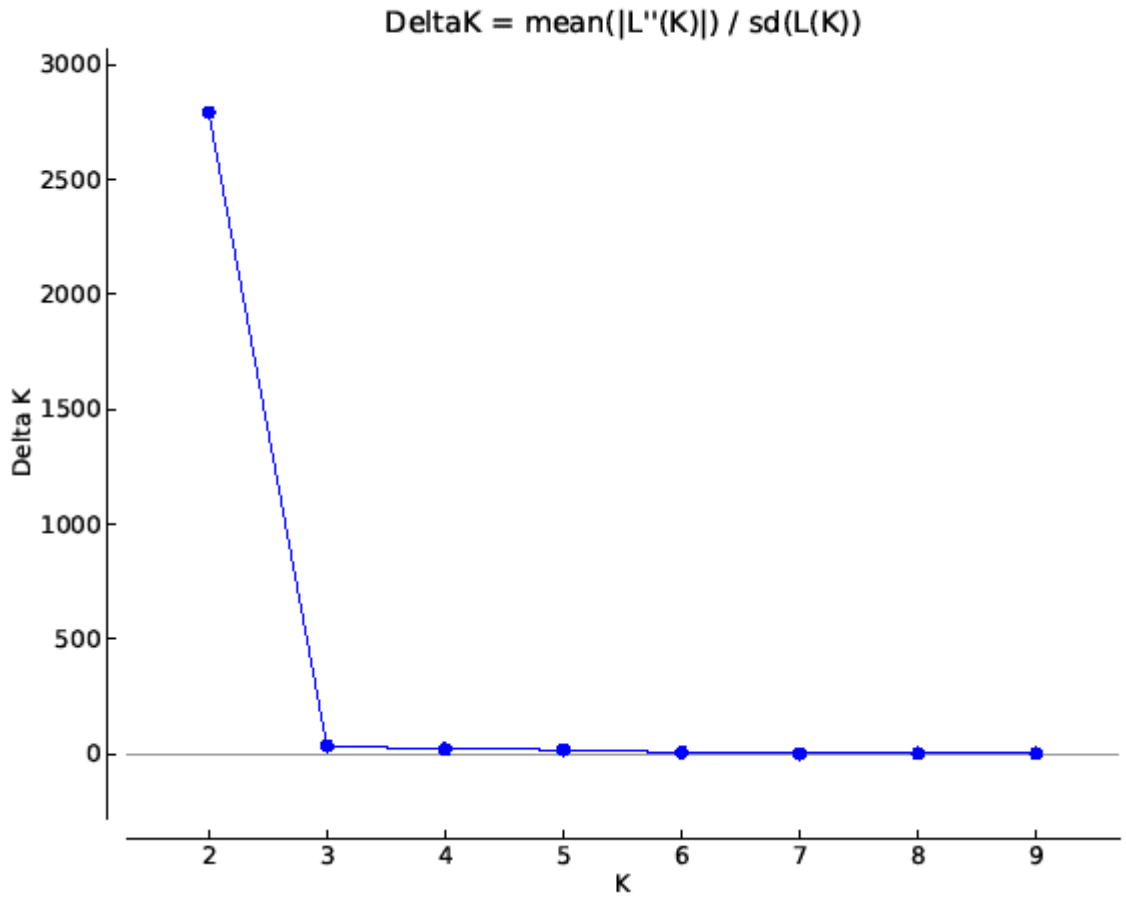

Figure S3, Delta K plot to determine the optimal number of genetic clusters

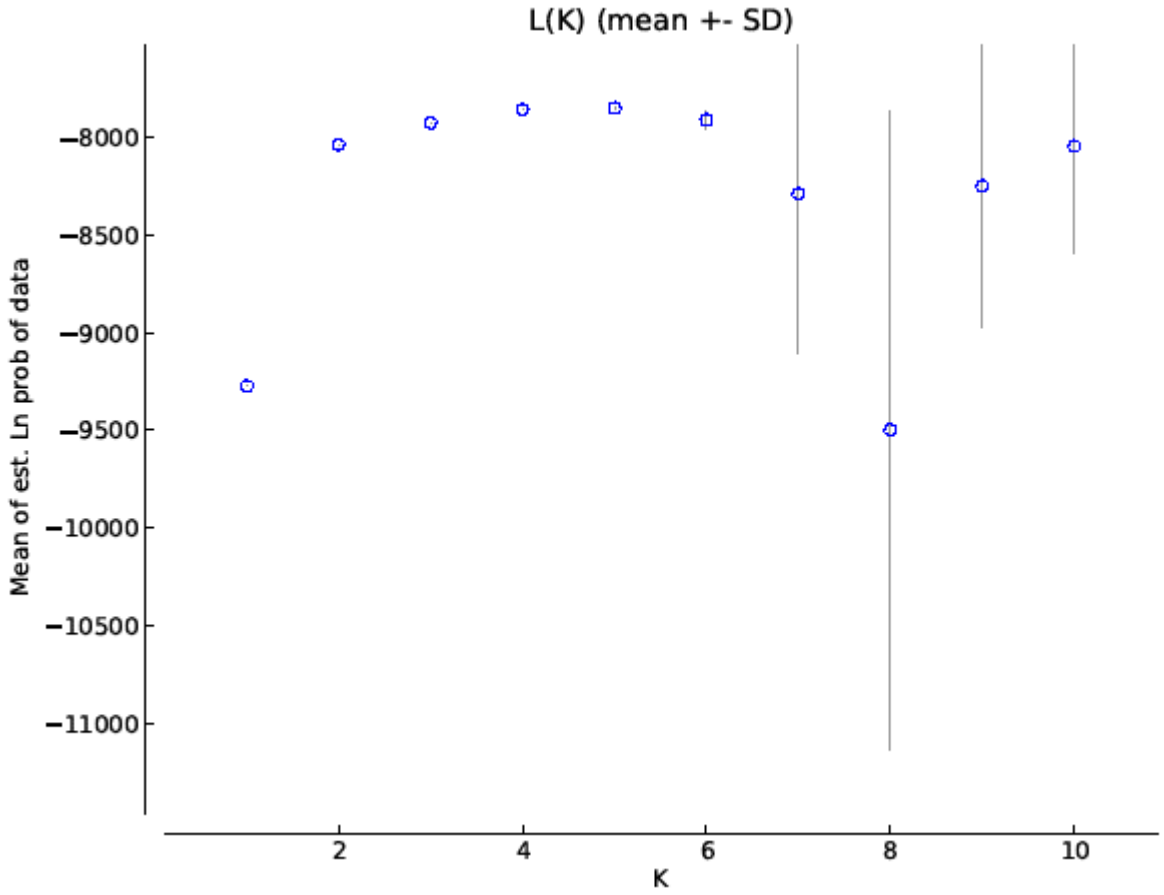

Figure S4, Mean Log probability plot to determine the optimal number of genetic clusters